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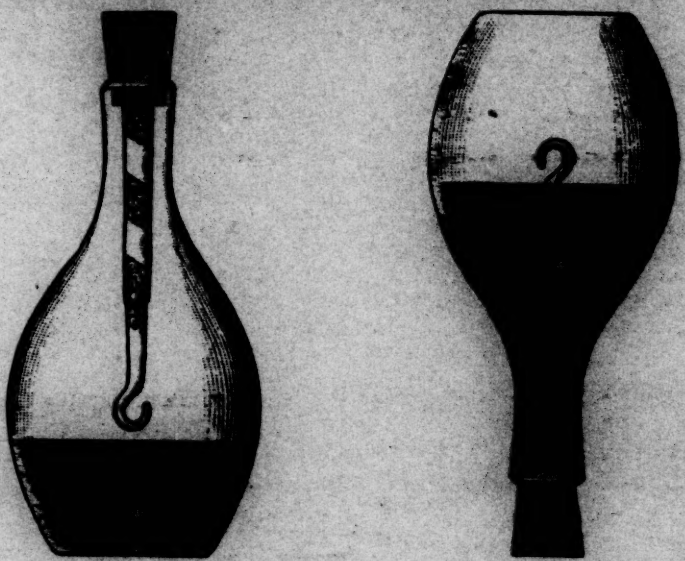

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NOTE ON THE FAT-SOLUBLE GROWTH-PROMOTING SUBSTANCE IN LARD AND COTTON-SEED OIL.

By AMY L. DANIELS and ROSEMARY LOUGHLIN.

(From the Department of Nutrition, Child Welfare Research Station, University of Iowa, Iowa City.)

(Received for publication, April 28, 1920.)

It is generally conceded by workers in the field of nutrition that the fat-soluble growth-promoting substance in certain fats, more particularly the extracted vegetable oils and lard, is present in such low concentration, if at all, that from the standpoint of nutrition these fats are of value merely as sources of energy. Indeed, there are reports in the literature of many feeding experiments where nutritive disaster on diets, which included vegetable oils or lard in fairly liberal amounts, have been averted by the substitution or addition of small amounts of those fats—butter fat, egg yolk fat, or cod liver oil—which are known to be rich in the fat-soluble vitamine.[1] The results with the vegetable oils and lard are not in all cases in accord with those which we have obtained in our laboratory; and we are led to believe that there may be some difference in the amount of the fat-soluble complex in commercial lards, and that certain vegetable oils may have values other than that of furnishing energy.

Since the relation between the fat-soluble vitamine and rickets is undergoing experimental scrutiny, our findings may be of interest in this connection. It is possible that recent workers[2] who have been using the vegetable oils, more particularly cotton-seed, in the study of rickets, with the thought that these are quite free from the fat-soluble complex may have been furnishing enough of the vitamine to meet the requirements of growth,

[1] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1913–14, xvi, 423; 1915, xx, 379; 1918, xxxiv, 17. McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1913, xv, 167; 1915, xxi, 179.

[2] Hess, A. F., *J. Biol. Chem.*, 1920, xli, p. xxxii.

especially if the experimental periods were of comparatively short duration.

In an investigation dealing with some problems in infant nutrition we had occasion to feed groups of rats on purified rations in composition simulating milk but which, we believed, contained the least possible amount of the fat-soluble hormone. These rations consisted of 18 per cent of casein, 28 per cent of fat, both lard and cotton-seed, respectively, 7 per cent of our salt mixture, 47 per cent of corn-starch, and the water-alcohol extract of 9 gm. of wheat embryo (cold process). In order to make sure that our experimental rations were as free as possible from the fat-soluble

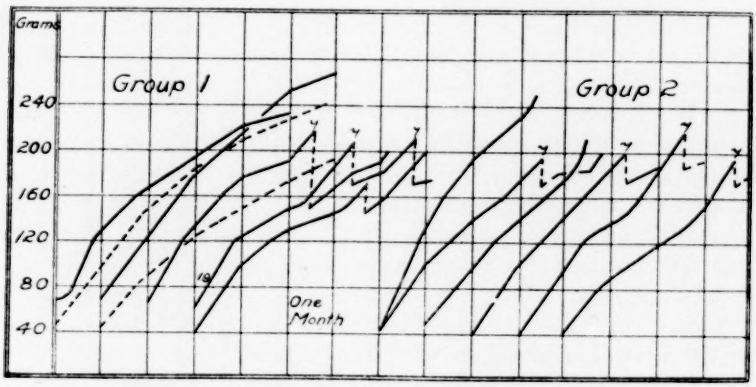


CHART 1. Both lard and cotton-seed oil apparently contain an appreciable amount of the fat-soluble vitamine. Animals fed rations consisting of casein 18 per cent, corn-starch 47 per cent, a suitable salt mixture, and 28 per cent of lard (Group 1) or cotton-seed oil (Group 2), respectively, together with an adequate amount of the water-alcohol extract of wheat embryo, grew normally, reproduced, and reared their young.

complex, both the casein and the wheat embryo, previous to the alcohol treatment, were extracted for 48 hours with ether ("Squibb, for anæsthesia") in a Soxhlet apparatus. The lard used was a commercial product rendered from the leaves and back fat. The cotton-seed oil was also a commercial product.[3]

Our animals on these rations have grown normally; in fact, their growth curves (Chart 1) are quite similar to those of control

[3] The cotton-seed oil was a product of the American Cotton Oil Company, New York.

animals fed a purified ration which included 5 per cent of butter fat in place of an equivalent amount of lard or cotton-seed oil, and in which neither casein nor the wheat embryo was extracted (Chart 2). All females in both lard and cotton-seed groups have reproduced and in certain instances second litters have been obtained. The young in both groups have been successfully reared on the diets of their mothers.

These results were so unexpected and so out of harmony with the published results of other workers that it was deemed of interest to determine the effects of a somewhat less amount of these two fats. Therefore rations in all other respects the same,

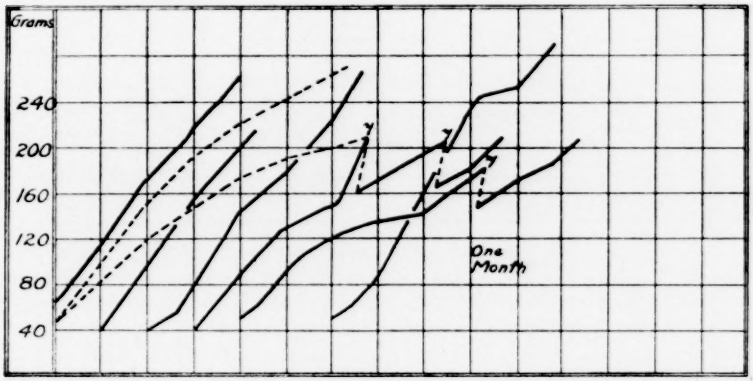


CHART 2. Curves of growth of control animals fed purified rations in which 5 per cent of butter was substituted for an equivalent amount of lard.

but containing 53 per cent of corn-starch and only 21 per cent of the fats under investigation, were fed. Since the food value of this mixture was 4.77 calories per gm. whereas that of our first mixture furnished 5.12 calories per gm., it was reasoned that the animals on this second mixture were getting about 6 per cent less fat than those receiving the 28 per cent fat ration.

On the lower fat food all the animals fed the lard mixture made normal growth gains for about 2 months, when the weight became stationary (Chart 3, Group 1). Those rats receiving the ration containing the lesser amount of cotton-seed oil (21 per cent) also grew far less well than those on the higher fat, the more vigorous

animals growing normally for only about 6 weeks, when there was a gradual decline in weight (Chart 3, Group 2).

These latter results are in accord with those of previous workers who have shown that both lard and cotton-seed oil contain much less of the fat-soluble vitamine than certain other fats. But, considering the fact that animals have made normal growth,

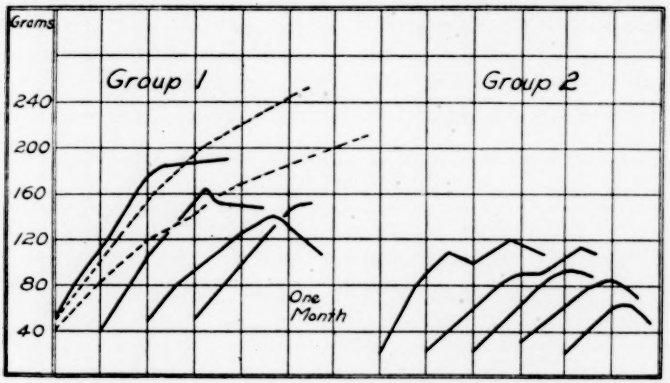


CHART 3. Animals fed purified rations furnishing 21 per cent of lard (Group 1) and cotton-seed oil (Group 2), respectively, failed to grow normally. Slightly better results were obtained with the lard than with the cotton-seed oil rations.

reproduced, and reared their young on diets in which the only apparent source of the vitamine was the lard or cotton-seed oil, it would seem that both these fats contain appreciable amounts of the fat-soluble growth stimulant. This, however, is demonstrable only when fairly large amounts are fed.

ACIDITY OF ASH-FREE AND OF COMMERCIAL GELATIN SOLUTIONS.

By H. E. PATTEN and T. O. KELLEMS.

(*From the Bureau of Chemistry, United States Department of Agriculture, Washington.*)

(Received for publication, May 4, 1920.)

In connection with measurements of the hydrogen ion concentration of various gelatin solutions,[1] it was thought desirable to compare the free acidity of ash-free gelatin with that of commercial gelatin. Accordingly, solutions were made up containing 0.5 per cent of ash-free[2] gelatin and different concentrations of hydrochloric acid (or of sodium hydroxide) and their hydrogen ion concentrations determined in each case electrometrically. The results obtained are given in Table I.

In this table the first column shows the normality of the gelatin solutions with respect to hydrochloric acid (or to sodium hydroxide). The second column shows the pH value.[3]

Similarly, solutions containing 0.5 per cent of commercial gelatin were made up, the acid alkali range of concentration being the same as for the solutions of ash-free gelatin, and the hydrogen ion concentration was measured. The results are given in Table II.

[1] Patten, H. E., and Johnson, A. J., *J. Biol. Chem.*, 1919, xxxviii, 179.

[2] The ash-free gelatin used in these experiments was kindly furnished by Mr. C. R. Smith of the Bureau of Chemistry.

[3] The hydrogen ion concentration was measured by a Clark electrode vessel, our electrode being of gold plated with paladium black immediately before each determination. The voltage was read on a Leeds and Northrup potentiometer of standard design, the temperature of the electrode cell and of the calomel half cell being maintained by an air thermostat, electrically controlled, at 25°C.

$$\text{pH} = \log \frac{1}{\text{concentration of hydrogen ion}}$$

Thus, a solution of 0.01 N with respect to hydrogen ions would have a pH = 2.

The results are presented graphically in Fig. 1. The exponent of the hydrogen ion concentration, pH, is taken as abscissa and the normality of the total system with respect to acid (or to alkali) as ordinate. To avoid excessive use of ciphers the negative exponent of the normality is used; thus, where the normality is 1×10^{-6} 6 is used. 1 does not mean normal but 0.1 normal. The ordinate thus extends from the highest concentration of acid through zero concentration of acid on through the regions of higher alkaline concentration.

TABLE I.

Normality with respect to HCl.	pH	Normality with respect to NaOH.	pH
10^{-1}	0.77	0.0	4.81
10^{-2}	1.96	10^{-6}	4.89
10^{-3}	3.89	10^{-5}	5.00
10^{-4}	4.69	10^{-4}	5.06
10^{-5}	4.74	10^{-3}	6.49
10^{-6}	4.83	10^{-2}	11.33

TABLE II.

Normality with respect to HCl.	pH	Normality with respect to NaOH.	pH
10^{-1}	0.96	0.0	5.6
10^{-2}	2.12	10^{-6}	5.54
10^{-3}	4.49	10^{-5}	5.66
10^{-4}	5.4	10^{-4}	5.69
10^{-5}	5.6	10^{-3}	8.78
10^{-6}	5.6	10^{-2}	11.57

It will be observed that the isoelectric point of ash-free gelatin (Smith), Curve 1, lies at pH = 4.8, corresponding to a hydrogen ion concentration of 1.59×10^{-5}, and that the isoelectric point of commercial gelatin, Curve 2, lies further toward the alkaline region and comes at pH = 5.64 (hydrogen ion concentration of 2.28×10^{-5}).

Ash determinations were made on both samples of gelatin used. The ash-free gelatin (Smith) showed on ignition 0.011 per cent residue which on treatment with hydrofluoric acid (to remove silica) and after a second ignition was completely volatil-

ized. A second portion of the gelatin was ignited and, without using hydrofluoric acid, the ash was treated with neutral distilled water. This solution showed no alkalinity with phenol red. The sample of commercial gelatin, however, showed a residue

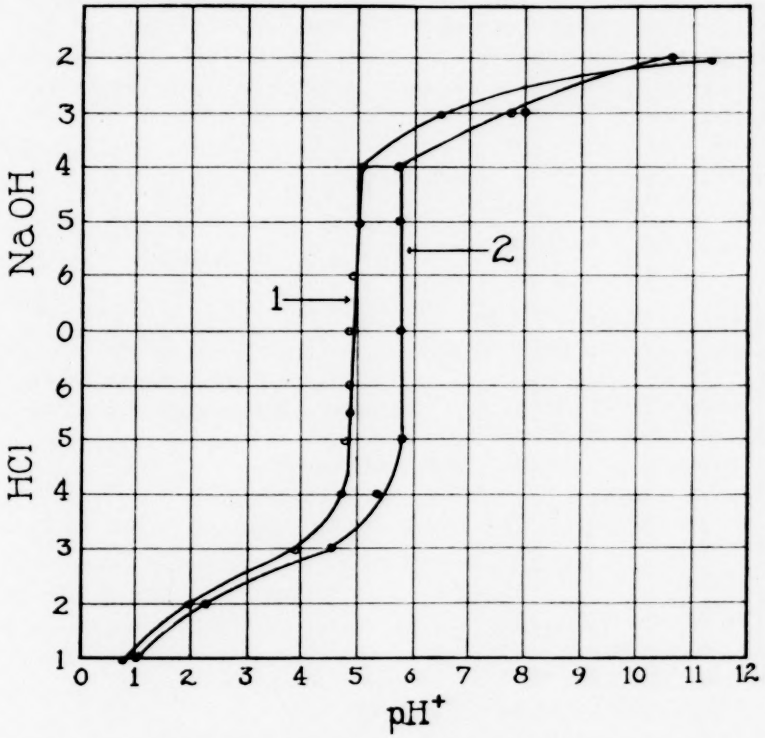


FIG. 1. Titration of 0.5 per cent gelatin in water at 25°C. Curve 1, ash-free gelatin (Smith). Curve 2, commercial gelatin. Ordinates, normality of acid (or alkali) in total system expressed as the negative index of normality; abscissæ are pH^+.

after the first ignition of 0.74 per cent. After being treated eight times with hydrofluoric acid this residue was reduced to 0.25 per cent of the original gelatin. A second portion of the commercial gelatin was ignited and the ash dissolved in neutral distilled water and titrated with standard HCl solution. This

showed an alkalinity corresponding to 0.000577 gm. of CaO per gm. of gelatin, or to a pH of 4.29 in a 0.5 per cent gelatin solution (hydrogen ion concentration of 5.14×10^{-5}), assuming it is all in solution.

It is evident that the displacement of the isoelectric point of commercial gelatin was due to the alkali content, part of the alkali being combined with the gelatin so that not all the alkali appearing in the ash is available to displace the hydrogen ion concentration.

The isoelectric point of ash-free gelatin (Smith) is identical with that of Loeb's so called isoelectric gelatin.[4]

[4] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 237.

SEDOHEPTOSE, A NEW SUGAR FROM SEDUM SPECTABILE. II.

By F. B. La FORGE.

(From the Bureau of Chemistry, United States Department of Agriculture, Washington.)

(Received for publication, March 30, 1920.)

In a previous publication[1] a new ketose was described possessing properties which seemed to place it in a unique position among all known members of the sugar group.

While the original compound reduced Fehling's solution strongly, it lost about 80 per cent of its reducing power on boiling with dilute acids.

When the transformed compound was treated with benzaldehyde in 50 per cent H_2SO_4 solution, a crystalline product was obtained, which proved to be the benzal derivative of a heptose that had lost 1 molecule of water. By hydrolysis of this benzal compound the anhydride itself ($C_7H_{12}O_6$) was obtained as crystalline anhydrosedoheptose. This compound did not reduce Fehling's solution. On heating with dilute acid the anhydride passed to the extent of about 20 per cent into the reducing sedoheptose ($C_7H_{14}O_7$) and there is thus an equilibrium in such solutions between the two substances.

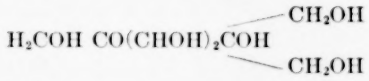
Upon reduction with sodium amalgam in neutral or slightly alkaline solution sedoheptose yielded two heptitols, which property, and also the fact that it was not oxidized by bromine, served to characterize the new sugar as a ketose.

Its tendency to form a non-reducing anhydride, which may be regarded also as an inner glucoside, is a property which served to differentiate the sugar of the sedum plant from all other members of this group.

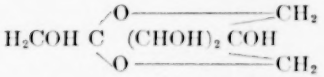
There are several considerations of structure which might offer some explanation of the abnormality displayed by sedo-

[1] La Forge, F. B., and Hudson, C. S., *J. Biol. Chem.*, 1917, xxx, 61.

heptose. One of these is the theory that sedoheptose might have a branch chain formula such as



The anhydride might then be represented by



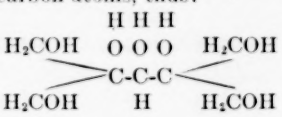
But there are two good reasons why the branch chain theory must be excluded.

In the first place, one of the heptitols obtained on reduction of sedoheptose is inactive, while all possible branch chain heptitols, according to theoretical considerations, are active.[2] Second, oxidation of a branch chain heptitol with HNO_3 should lead to a tribasic acid, while experiment showed that a dibasic, pentoxypimelic acid was obtained on oxidation of sedoheptitol.

Oxidation of α-Sedoheptitol.

18 gm. of α-sedoheptitol[1] were oxidized according to the method of Fischer by warming with three parts of HNO_3 (specific gravity 1.2). After standing for 3 or 4 hours at about 50° the solution was rapidly concentrated on the steam bath and most of the excess acid expelled by repeated evaporation with water. The reaction product was finally boiled with calcium carbonate for several hours and filtered. The filtrate slowly deposited crystals which were collected and purified by dissolving in water containing a slight excess of oxalic acid and again transforming the acid into the calcium salt. The solution yielded the salt as pure white crystals. The yield was 3 gm.

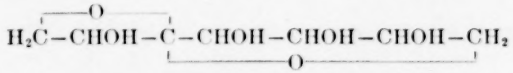
[2] It is, to be sure, possible to write a formula for an inactive heptitol having no asymmetric carbon atoms, thus:



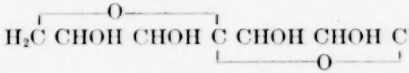
0.1898 gm. substance dried at 100° gave 0.2132 gm. CO_2, 0.0539 gm. H_2O, and 0.0372 gm. CaO.

	Calculated for Ca $C_7H_{10}O_9$ (278).	Found.
	per cent	*per cent*
Ca	14.38	14.00
C	30.21	30.60
H	3.59	3.15

These results agree for the Ca salt of pentoxypimelic acid. Therefore, sedoheptitol and, consequently, sedoheptose in all probability have a straight chain formula. The three possible structures for straight chain, seven carbon ketoses are: alpha, $CH_2OH\ CO\ CHOH\ CHOH\ CHOH\ CHOH\ CH_2OH$; beta, $CH_2OH\ CHOH\ CO\ CHOH\ CHOH\ CHOH\ CHOH\ CH_2OH$; and gamma,[3] $CH_2OH\ CHOH\ CHOH\ CO\ CHOH\ CHOH\ CH_2OH$. Of these three possibilities beta and gamma might explain the formation of an anhydride as follows. Beta (by elimination of water)



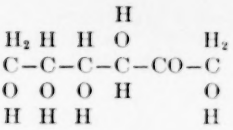
and gamma (by elimination of water)



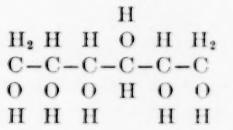
As has already been stated two heptitols have been obtained from sedoheptose by reduction; one, α-sedoheptitol, is active, the other, β-sedoheptitol, is inactive.

It will readily be seen that if the space formulas of these heptitols were known the configuration and also the structure of sedoheptose would follow, because the two heptitols would differ only in having the position of one hydroxyl reversed. The carbon atom on which this reversal took place would be the one which originally carried the carbonyl group in the ketose from which the two heptitols were derived. Thus, in the case of fructose,

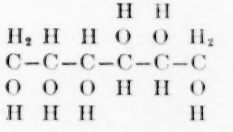
[3] The words alpha, beta, and gamma are written out where they refer to the position of carbon atoms in the molecule. The Greek letters α and β are used to distinguish between epimeric compounds.



the two resulting hexitols, sorbitol



and mannitol

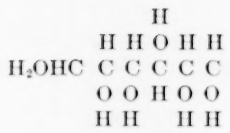


have the hydroxyl groups reversed in the alpha position and the formula of the sugar would be evident if only the configuration of sorbitol and mannitol were known. The same method could be applied to sedoheptose.

There are according to the theory ten heptitols, if optical isomerism is disregarded. Six of these are optically active and four are inactive. Their space formulas are as follows.

Heptitols.

From Glucose.



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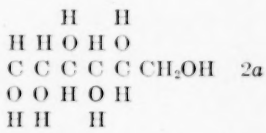
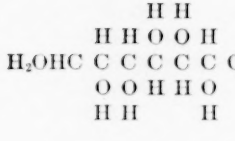


2*a*

From Mannose.



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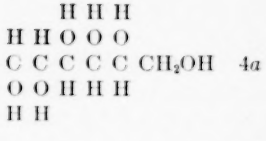
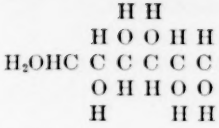


4*a*

From Galactose.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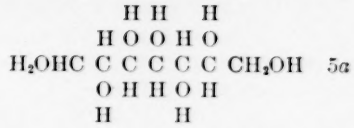
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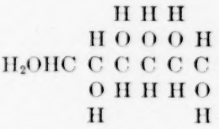
From Talose.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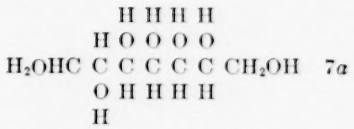
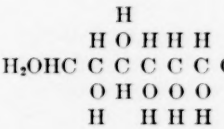
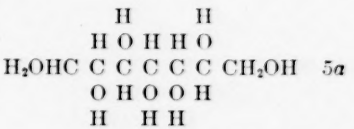
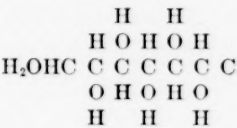

7*a*

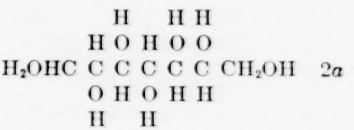
From Gulose.


8*a*

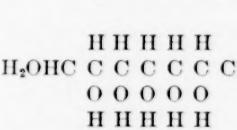

5*a*

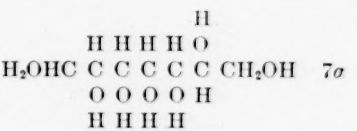
From Idose.


9*i*

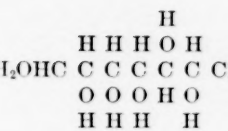

2*a*

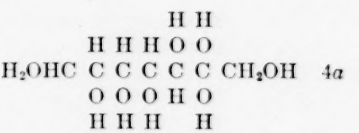
From Allose.


10*i*


7*a*

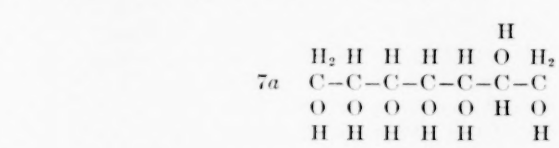
From Altrose.


8*a*


4*a*

Those which are active are designated *a*, those which are inactive, *i*. Of the active heptitols all but 7*a* are known while 1*i* is the only known inactive one.

Neither of the heptitols from sedoheptose is identical with any of the known heptitols. But since one of them, α-sedoheptitol, is active it must have the formula



because this is the only remaining possibility according to the theory.

The inactive β-sedoheptitol might have one of three possible configurations, 6*i*, 9*i*, or 10*i*, since 1*i* is known and is excluded on account of the properties of its acetyl and benzal derivatives.[4]

Returning to the consideration of the three possible structures, alpha, beta, and gamma of sedoheptose, we may exclude the last named possibility for the reason that the gamma position in the case of a seven carbon chain is in the middle of the molecule. From this it follows that if the keto group in the original sugar was in this position, and if it yielded on reduction two heptitols, one of which was inactive, the second heptitol which resulted from this treatment would also have to be inactive. Therefore, the gamma keto structure is excluded.

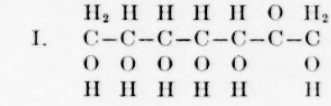
There remain the alpha and beta structures to be considered.

The latter structure might explain the abnormal properties of sedoheptose, but the properties of α- and β-sedoheptitol are not in agreement with theoretical requirements. Inactive β-sedoheptitol must have one of the configurations 6*i*, 9*i*, or 10*i* and α-sedoheptitol must differ in having the position of one hydroxyl in the beta position reversed. With this change 1*i* and 6*i* would yield 3*a* or α-mannoheptitol of M. P. 180° and could not be confused with active α-sedoheptitol. Likewise, with the same change both 9*i* and 10*i* would yield 8*a* (β-guloheptitol of M. P.

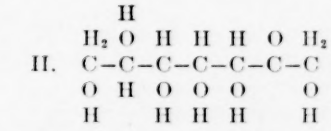
[4] Fischer, E., *Ann. Chem.*, 1892, cclxx, 64. Wherry, E. T., *J. Biol. Chem.*, 1920, xlii, 377.

128°).[5] Thus, the beta possibility must also be excluded and there remains only the alpha structure to be considered.

There are two possible configurations for an alpha keto structure which will meet the conditions in the case of sedoheptose.



and



[5] La Forge, F. B., *J. Biol. Chem.*, 1920, xli, 251.

β-Glucoheptitol 2*a* and β-guloheptitol 8*a* both melt at about the same temperature as β-sedoheptitol and all three show little or no optical activity. It is not possible, however, that β-sedoheptitol could be identical with either, as can be shown by theoretical as well as experimental considerations. The space formulas on pages 370 and 371 will show that neither 2*a* nor 8*a* could lead to 7*a*, the only remaining unknown active heptitol by the reversal of the position of one hydroxyl group.

In the following table the melting points of the three compounds in question are given together with their optical rotations.

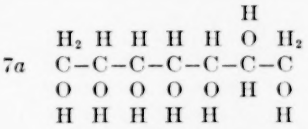
	Melting point. °C.	$[\alpha]_D$
β-Glucoheptitol	130–131	+0.8
β-Guloheptitol	129	0
β-Sedoheptitol[1]	128	0
β-Gluco- + β-sedoheptitol	119–120	
β-Gulo- + β-sedoheptitol	121	
β-Gluco- + β-guloheptitol	118–120	

In addition to these differences β-glucoheptitol forms a characteristic gelatinous benzal derivative. Dr. Edgar T. Wherry of this Bureau has measured the refractive indices of the three substances with the following results.

	α	β	γ	γ-α
β-Glucoheptitol	1.542	1.550	1.552	0.010
β-Guloheptitol	1.565	1.570	1.586	0.021
β-Sedoheptitol	1.564	1.570	1.584	0.020

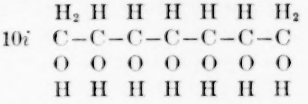
The differences brought out between β-glucoheptitol serve to differentiate it sharply from the other two. The figures for β-guloheptose and β-sedoheptose, however, do not show sufficient differences to allow a conclusion to be drawn as to their separate identity. This result is not at all surprising since the last mentioned compounds differ only in the configuration of their molecules by the position of one hydroxyl group.

Both of these configurations will give on reduction the active heptitol



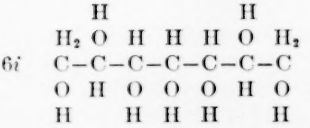
7a

and both will give unknown inactive heptitols



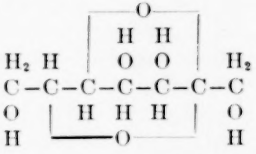
10i

in the case of I and



6i

in the case of II. Any two of the five hydroxyl groups might be involved in the formation of the anhydride, which might have, for instance, the formula



It is not possible with our present knowledge to decide between I and II and before this question can be settled the active heptitol, *7a*, and one of the inactive ones, *10i* or *9i*, will have to be prepared. For the preparation of these compounds considerable amounts of talose or allose would be necessary, and until this material is available the decision between the two possible formulas for sedoheptose will have to be withheld.

The formulas for sedoheptose, one of which one seems forced to accept, do not offer any explanation for the abnormal behavior of this sugar toward dilute acids. The only peculiarity in the case of I is in the hydroxyl groups, all being on one side of the molecule. Whether such a circumstance can, in general, be the cause of such a great difference in properties only further study of the higher sugars can decide.

VOLEMITE.

By F. B. La FORGE.

(From the Bureau of Chemistry, United States Department of Agriculture, Washington.)

(Received for publication, January 21, 1920.)

In a previous article on sedoheptose[1] reference was made to the natural heptitol, volemite. This compound seemed to agree in its properties with those of α-sedoheptitol, one of the heptitols obtained on reduction of sedoheptose with sodium amalgam. The identity of the two compounds would have been accepted but for a discrepancy in the melting points of tribenzal α-sedoheptitol and that recorded by Bougault and Allard[2,3] for the corresponding derivative of volemite.

Since the publication of the article on sedoheptose, I have received, through the courtesy of Professor Emile Bourquelot of Paris, a sample of natural volemite and also a statement that the recorded melting point of tribenzal volemite was erroneous.

The sample furnished by Professor Bourquelot, after crystallization from 75 per cent alcohol, melted at 151° uncorrected. This is the melting point which has been accepted for α-sedoheptitol. The two products, when mixed, also melted at 151° uncorrected.

An optical examination of crystals of the two substances failed to disclose any differences.

For further comparison, the acetal of α-sedoheptitol has been prepared.

Acetal of α-sedoheptitol.—1.4 gm. of α-sedoheptitol in 3 cc. of 50 per cent sulfuric acid were shaken with 3 gm. of acetaldehyde and the reaction mixture was allowed to stand over night in the

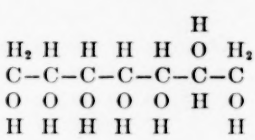
[1] La Forge, F. B., and Hudson, C. S., *J. Biol. Chem.*, 1917, xxx, 61.

[2] Bougault, J., and Allard, G., *Compt. rend. Acad.*, 1902, cxxxv, 796.

[3] In general the benzal derivatives of the higher alcohols are not well adapted for identification purposes as they often show little difference in melting point. In the article on sedoheptose the melting point of tribenzal sedoheptitol was given as 190°. This should be corrected to 225°.

ice box. The resulting mass of crystals was washed with water and twice recrystallized from a small amount of 75 per cent alcohol. The compound melted at 191–194° uncorrected which agrees with the melting point of volemite acetal recorded by Bougault and Allard.[2] 0.5544 gm. in 25 cc. of chloroform rotated in a 2 dm. tube −2.60° to the left $(\alpha)_D = -45.55$. The authors record $(\alpha)_D$ for the volemite derivative as −46.40 in chloroform solution. There can, therefore, be no further doubt as to the identity of the two heptitols.

As shown in the preceding publication,[4] the space formula



or its mirror image must be accepted as corresponding to α-sedoheptitol and hence must also represent the configuration of volemite.

[4] La Forge, F. B., *J. Biol. Chem.*, 1920, xlii, 367.

OPTICAL PROPERTIES OF A SERIES OF HEPTITOLS.

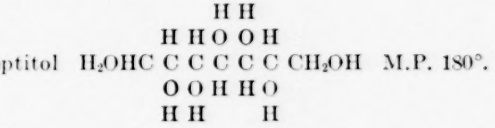
By EDGAR T. WHERRY.

(From the Bureau of Chemistry, United States Department of Agriculture, Washington.)

(Received for publication, May 5, 1920.)

Eight of the ten possible heptitols were prepared by Dr. F. B. La Forge of the Bureau of Chemistry and turned over to the writer for investigation.

Optical studies were made of them by the immersion method under the polarizing microscope, using oily immersion liquids of known refractive indices, and light transmitted through a combination of two color screens with a maximum transmission at D. As it seemed of interest to obtain the refractivities, determinations of specific gravity were also made, by suspending the materials in mixtures of carbon tetrachloride and benzene, and measuring the specific gravity of the liquid in each case with a Westphal balance. The n^2 formula[1] was used for calculating refractivity; the theoretical value, using Eisenlohr's data, C, 2.418, H, 1.100, and O, 1.525, should be, for $C_7H_{16}O_7$, 45.20. The results of these observations are here presented.



Described by Maquenne.[2]

In ordinary light: seen to consist of minute needles and rods. Refractive indices (D):

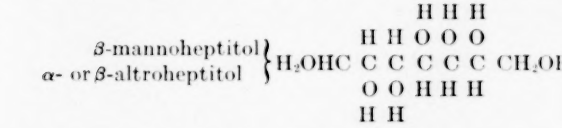
$\alpha = 1.538$, $\beta = 1.545$, $\gamma = 1.549$, $\gamma - \alpha = 0.011$, all $\pm$ 0.005; index α is often shown lengthwise. *In parallel polarized light, nicols*

[1] $M = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{W}{\rho}$, where n is the mean refractive index for D, W, the molecular weight (212.13), and ρ, the density of the substance.

[2] Maquenne, *Compt. rend. Acad.*, 1888, cvii, 583.

crossed: Birefringence moderate, the colors being first order; extinction parallel; elongation −.

Specific gravity = 1.485; mean n = 1.544; refractivity = 45.10.



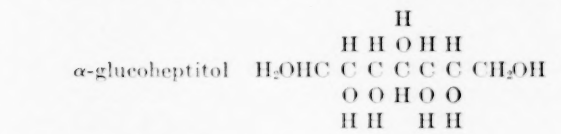
M.P. 125°.

Described by Peirce;[3] prepared from mannose.

In ordinary light: seen to consist of minute needles, often radiating. Refractive indices (D):

α = 1.533, β = (?), γ = 1.545, $\gamma - \alpha$ = 0.012, all ± 0.005; index γ is often shown lengthwise. *In parallel polarized light, nicols crossed:* Birefringence moderate, the colors being first order; extinction parallel; elongation +.

Specific gravity = 1.470; mean n = 1.539; refractivity = 45.21.



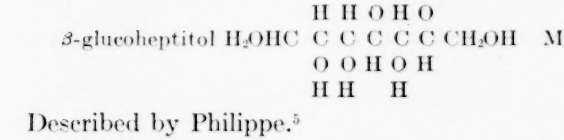
M.P. 128°.

Described by Fischer.[4]

In ordinary light: seen to consist of pointed rods, sometimes radiating. Refractive indices (D):

α = 1.548, β = 1.550, γ = 1.558, $\gamma - \alpha$ = 0.010, all ± 0.003; intermediate values are usually shown. *In parallel polarized light, nicols crossed:* Birefringence moderate, the colors being first order; extinction inclined, reaching a maximum angle of 40°, although parallel extinguishing rods are not uncommon; elongation variable. *In convergent polarized light, nicols crossed:* a partial biaxial interference figure occasionally shown; 2 E large, and sign +.

Specific gravity = 1.520; mean n = 1.552; refractivity = 44.60.



M.P. 128–129°.

Described by Philippe.[5]

[3] Peirce, G., *J. Biol. Chem.*, 1915, xxiii, 334.

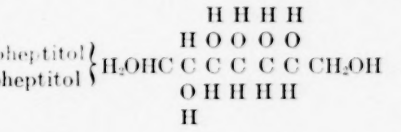
[4] Fischer, E., *Ann. Chem.*, 1892, ccIxx. 64.

[5] Philippe, L. H., *Ann. chim. phys.*, 1912, xxvi, 289.

In ordinary light: seen to consist of plates and rods, the latter radiating. Refractive indices (D):
$\alpha = 1.542$, $\beta = 1.550$, $\gamma = 1.552$, $\gamma - \alpha = 0.010$, all ± 0.003; index γ is usually shown lengthwise, and β is often shown on the plates. *In parallel polarized light, nicols crossed:* Birefringence moderate, the colors being first order; extinction parallel; elongation +.

Specific gravity = 1.510; mean $n = 1.548$; refractivity = 44.61.

α-sedoheptitol
α-or β-taloheptitol



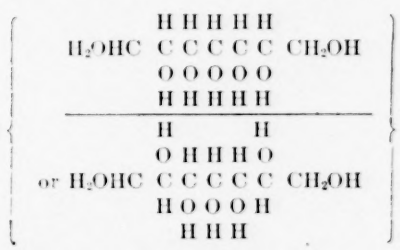
M.P. 151°.

Described by La Forge and Hudson;[6] prepared from sedoheptose.

In ordinary light: seen to consist of rods and needles, often bunched. Refractive indices (D):
$\alpha = 1.550$, $\beta = 1.555$, $\gamma = 1.562$, $\gamma - \alpha = 0.012$, all ± 0.003; index γ is often shown lengthwise. *In parallel polarized light, nicols crossed:* Birefringence moderate, the colors being first order; extinction parallel; elongation +.

Specific gravity = 1.520; mean $n = 1.556$; refractivity = 44.86.

β-sedoheptitol



M.P. 128°.

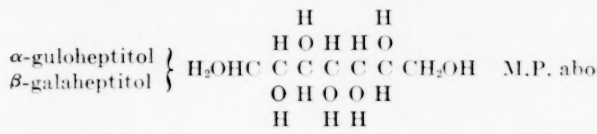
Described by La Forge and Hudson.[7]

In ordinary light: seen to consist of plates and rods, with 120° angles. Refractive indices (D) (checked by a monochromatic illuminator):
$\alpha = 1.564$, $\beta = 1.570$, $\gamma = 1.584$, $\gamma - \alpha = 0.020$, all ± 0.002; index α is often shown lengthwise. *In parallel polarized light, nicols crossed:* Birefringence moderately strong, the colors being

[6] La Forge, F. B., and Hudson, C. S., *J. Biol. Chem.*, 1917, xxx, 68.
[7] La Forge and Hudson,[6] p. 71.

first to second order; extinction parallel; elongation mostly −. *In convergent polarized light, nicols crossed:* a partial biaxial interference figure occasionally shown; 2 E large, and sign +.

Specific gravity = 1.590; mean n = 1.573; refractivity = 43.95.

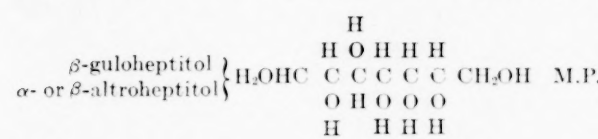


Described by Peirce[3] and by La Forge;[8] prepared from gulose.

In ordinary light: seen to consist of irregular grains. Refractive indices (D):

α = 1.554, β = 1.560, γ = 1.570, $\gamma - \alpha$ = 0.016, all ± 0.003; means of these values are usually shown. *In parallel polarized light, nicols crossed:* Birefringence moderate, the colors being first to second order; extinction apparently inclined; elongation mostly −. *In convergent polarized light, nicols crossed:* A partial biaxial interference figure frequently shown; 2E moderately large, and sign +.

Specific gravity = 1.560; mean n = 1.561; refractivity = 44.03.



Described by La Forge;[9] prepared from gulose.

In ordinary light: seen to consist of pointed rods. Refractive indices (D) (checked by a monochromatic illuminator):

α = 1.565, β = 1.570, γ = 1.586, $\gamma - \alpha$ = 0.021, all ± 0.002; means of these values are usually shown. *In parallel polarized light, nicols crossed:* Birefringence moderately strong, the colors being first to second order; extinction somewhat inclined; elongation variable. *In convergent polarized light, nicols crossed:* A partial biaxial interference figure frequently shown; 2E large, and sign +.

[8] La Forge, F. B., *J. Biol. Chem.*, 1920, xli, 254.

[9] La Forge,[8] p. 256.

TABLE I.

Properties of the Eight Heptitols Studied.

Substance	α-manno-heptitol.	β-manno-heptitol.	α-gluco-heptitol.	β-gluco-heptitol.	α-sedo-heptitol. (?)-talo-heptitol.	β-sedo-heptitol.	α-gulo-heptitol. β-gala-heptitol.	β-gulo-heptitol. (?)-altro-heptitol.
Habit	Minute needles.	Radiating needles.	Pointed rods.	Plates, rods.	Rods, needles.	Plates, rods.	Grains.	Pointed rods.
Refractive indices α	1.538	1.533	1.548	1.542	1.550	1.564	1.554	1.565
β	1.545	(?)	1.550	1.550	1.555	1.570	1.560	1.570
γ	1.549	1.545	1.558	1.552	1.562	1.584	1.570	1.586
Birefringence γ-α	0.011	0.012	0.010	0.010	0.012	0.020	0.016	0.021
Usually shown	α	γ	Means.	γ	γ	α	Means.	Means.
Birefringence	Moderate.	Moderate.	Moderate.	Moderate.	Moderate.	Strong.	Moderate.	Strong.
Order of colors	1	1	1	1	1	1-2	1-2	1-2
Extinction	Parallel.	Parallel.	40°	Parallel.	Parallel.	Parallel.	Inclined.	Inclined.
Elongation	−	+	±	+	+	−	−	±
Interference figure			Occasional.			Occasional.	Often.	Often.
Axial angle, 2E			Large.			Large.	Large.	Large.
Optical character	−	(?)	+	−	+	+	+	+
Mean *n*	1.544	1.539	1.552	1.548	1.556	1.573	1.561	1.574
Specific gravity	1.485	1.470	1.520	1.510	1.520	1.590	1.560	1.585
Refractivity	45.10	45.21	44.60	44.61	44.86	43.95	44.03	44.18

Specific gravity = 1.585; mean n = 1.574; refractivity = 44.18.

Discussion of Results.—It is noteworthy that the heptitols studied show in nearly every case highly distinctive optical properties, so that they could be differentiated unmistakably by this means. In the one instance of close resemblance, namely β-sedo- and β-guloheptitols, the differences between their respective α and γ values are too slight to enable them to be told apart except by the most careful work; but with a monochromatic illuminator this should be possible.

The refractivity of most of these substances comes out rather lower than the theoretical value; this must apparently be ascribed to some feature of their molecular configuration, but the exact nature of the effect is unknown.

INFLUENCE OF DIET ON THE ANTISCORBUTIC POTENCY OF MILK.*

By E. B. HART, H. STEENBOCK, and N. R. ELLIS.

(From the Department of Agricultural Chemistry, University of Wisconsin, Madison.)

PLATE 4.

(Received for publication, May 8, 1920.)

When Funk in 1914[1] indicated that there probably was a specific vitamine responsible for the prevention of scurvy, he expressed the belief that the diet of the milk-producing animal might be a factor in the relative antiscorbutic potency of the milks produced. This opinion was concurred in by Barnes and Hume,[2] and in a recent publication[3] we accepted this view as a possibility and a field for research. A preliminary note by Dutcher, Pierson, and Biester[4] on the same problem would indicate that they were in possession of facts tending to support this view.

There appears to be positive experimental evidence that the mammary gland does not have the power to synthesize the antineuritic vitamine and that its concentration in the milk is dependent on the supply of it in the diet.[5] However, from a recent publication by Osborne and Mendel[6] one would infer that there

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station, Madison.

[1] Funk, C., Die Vitamine, Wiesbaden, 1914.

[2] Barnes, R. E., and Hume, E. M., *Biochem. J.*, 1919, xiii, 306; *Lancet*, 1919, ii, 323.

[3] Hart, E. B., Steenbock, H., and Smith, D. W., *J. Biol. Chem.*, 1919, xxxviii, 305.

[4] Dutcher, R. A., Pierson, E. M., and Biester, A., *Science*, 1919, l, 184.

[5] Andrew, V. L., *Philippine J. Sc., Series B*, 1912, vii, 67. McCollum, E. V., Simmonds, N., and Pitz, W., *J. Biol. Chem.*, 1916, xxvii, 33. Steenbock, H., Kent, H. E., and Gross, E. G., *J. Biol. Chem.*, 1918, xxxv, 61.

[6] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1920, xli, 549.

is no difference in the water-soluble vitamine content of milk produced under winter feeding and summer pasture feeding conditions. This is not surprising when we recall the comparative stability of this vitamine and the probability that it is not destroyed in the drying of farm crops. But their experiment in itself does not decide that there would be no variation in the water-soluble vitamine content of milk if there were a wide variation in its concentration in any two rations compared. In respect to the fat-soluble or antixerophthalmic vitamine and its relation to diet concentration and abundance in milk there are no conclusive experimental data at present. Should it be found that this vitamine is generally associated or identical with certain yellow pigments of plant tissue[7] then there is abundant evidence that the concentration of this vitamine in the milk will be related to its concentration in the ration or diet.

EXPERIMENTAL.

We had excellent opportunity for studying this problem of dietary relation to the antiscorbutic vitamine concentration in milk. The University has maintained for the purpose of studying restricted ration problems a herd of eighteen cows, all of which are kept year after year on air-dried roughages and grains. No fresh green vegetable tissue of any kind has ever been fed this herd. Further, it is recognized through the results of recent investigations that the antiscorbutic properties of green plant tissue are greatly reduced in quantity even through the process of air-drying such as is customarily followed in the handling of farm crops. Such forage as finely ground alfalfa hay or corn stover[3] has been so reduced in its content of the antiscorbutic vitamine as to fail to prevent the development of scurvy in the guinea pig. Our results show that the milk from this herd was distinctly inferior in its content of the antiscorbutic vitamine to the milk derived from cows receiving a part of their ration as *summer pasture*, which consisted of fresh green material. The summer pasture milk was derived from cows of the University herd which during part of the day grazed on a timothy, blue

[7] Steenbock, H., *Science*, 1919, l, 352.

grass, clover pasture. The amount of green plant tissue consumed by these animals was, of course, unknown.

These differences in the antiscorbutic properties of milk are illustrated in Charts 1 to 8 inclusive. We used as a basal ration for the guinea pigs a mixture of heated alfalfa hay (finely ground), rolled oats, and common salt. The hay was heated in an autoclave for $\frac{1}{2}$ hour at 15 pounds pressure in order to reduce further its concentration of the antiscorbutic. This ration presumably furnished a sufficiency of all the dietary factors with the exception of an ample content of the antiscorbutic vitamine. For improvement of the protein mixture, where liquid milk was omitted, five

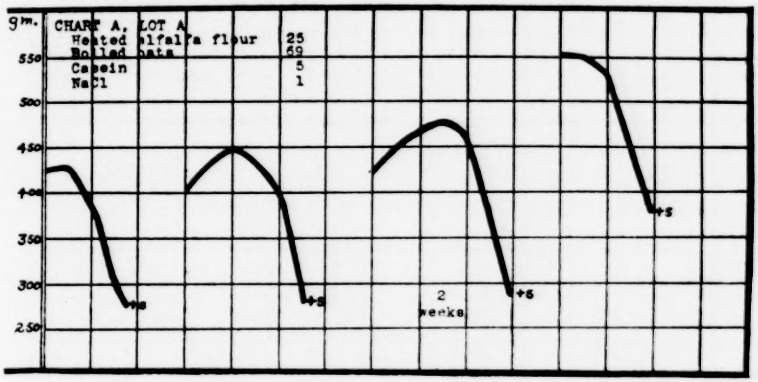


CHART A. Showing the early development of scurvy on the basal ration used in all our experiments.

parts of casein were added. Chart A illustrates the deficiency of this ration in the antiscorbutic vitamine. The fact that nutritional failure resulted even in the presence of casein is positive proof that nutritional failure in the presence of a liquid milk addition was not due to the inclusion in the diet of too little protein of efficient supplementing character.

Milk was given at different levels varying from 15 to 50 cc. per individual daily in the case of the summer pasture milk and from 15 to 100 cc. per individual in the case of the dry feed milks. It should be noted (Chart 1) that 15 cc. daily of summer pasture milk delayed the onset of scurvy, although not preventing its eventual occurrence in two of the animals of this group. One

animal, No. 2, apparently was fully protected against scurvy by but 15 cc. of such milk. This fact must emphasize not only variations of susceptibility in individuals, but the impossibility

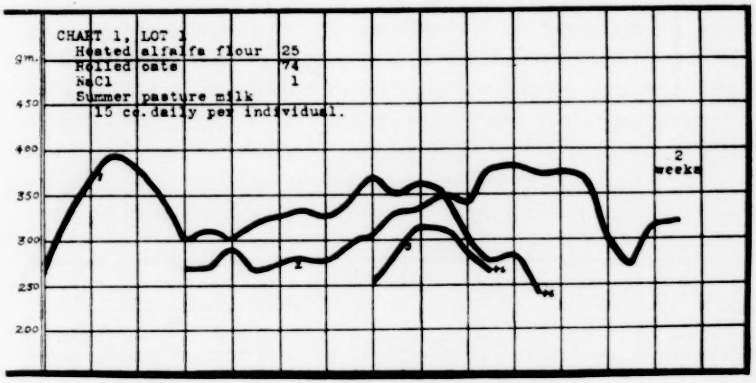


CHART 1. Showing the protection against scurvy by 15 cc. per individual daily of summer pasture milk. This amount of milk was too small for complete protection of all individuals against the disease.

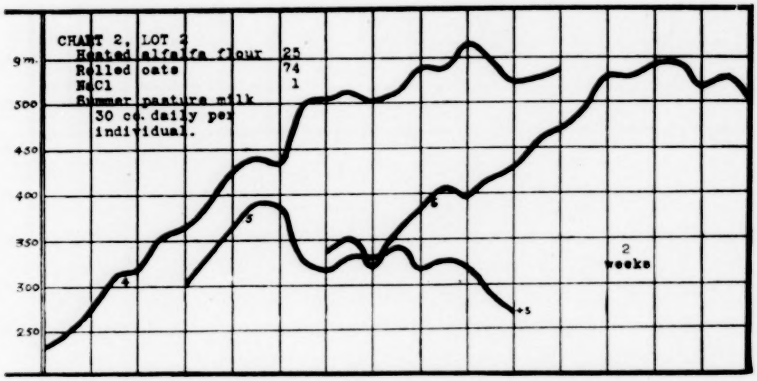


CHART 2. Showing the fuller protective action of 30 cc. per individual daily of summer pasture milk. This amount protected two animals completely, while one animal was not protected.

of fixing the minimum quantity of milk necessary for the protection of a 250 gm. guinea pig against scurvy. Where 30 cc. of summer pasture milk were allowed (Chart 2), but one animal in a

group of three developed scurvy. With 50 cc. of this type of milk consumed daily per individual none of the group developed scurvy (Chart 3). One animal from this lot, No. 9, died after 9

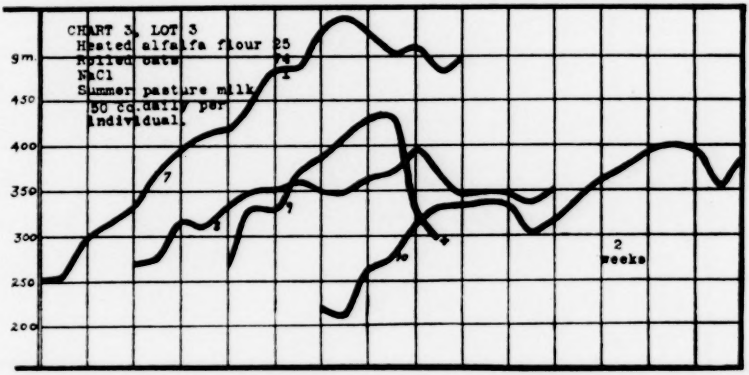


CHART 3. Showing the full protection against scurvy of 50 cc. of summer pasture milk when allowed daily per individual and used with a basal ration of rolled oats and heated alfalfa.

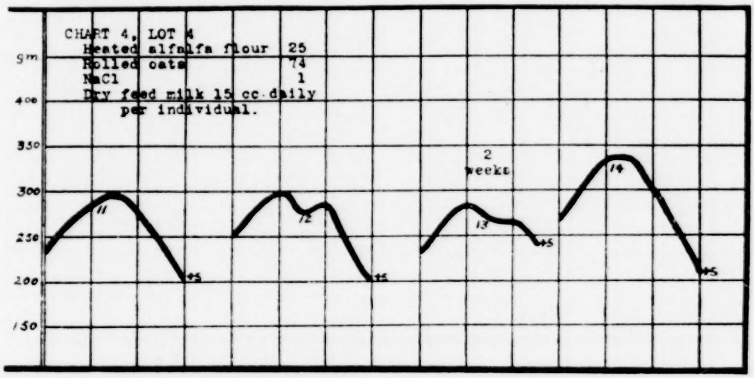


CHART 4. Showing the complete failure of 15 cc. daily per individual of dry feed milk to protect against scurvy. The basal ration was the same as in all other experiments.

weeks restriction to this ration, but not from scurvy; the cause of death was undetermined. On all these rations the amount of milk allowed was completely consumed.

Entirely different results were secured with the dry feed milk. This milk was distinctly inferior in its antiscorbutic properties to the summer pasture milk. No protection against scurvy was

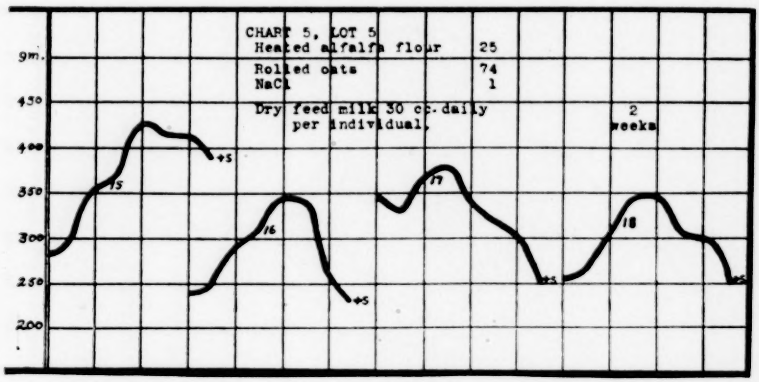


CHART 5. This chart shows that 30 cc. of dry feed milk daily per individual was insufficient as a source of the antiscorbutic vitamine.

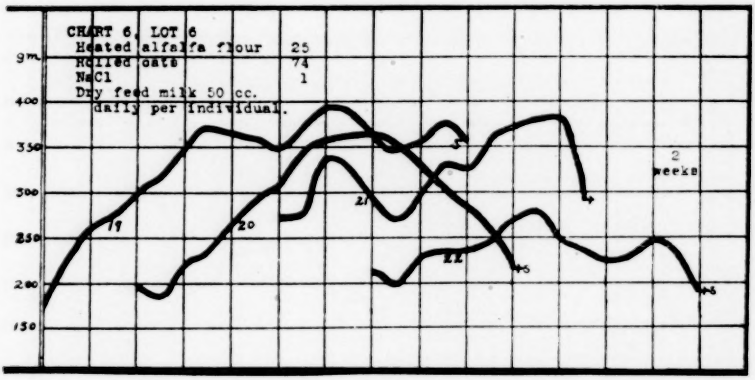


CHART 6. Not until 50 cc. of dry feed milk were allowed daily per individual was some protection against scurvy afforded. That amount only delayed the disease and did not prevent it.

afforded by a daily consumption per individual of 15 cc. (Chart 4), and twice that amount, or 30 cc. of milk, was little better as a preventative, if judgment can be based on the time elapsing before the development of the disease (Chart 5). With 50 cc. of this

milk daily per individual (Chart 6), there manifestly was some protection, but the fatality from scurvy was only delayed and not prevented. All but one of this group died with pronounced scurvy symptoms, but only after 12 to 14 weeks confinement to the ration. No. 21 of this group died after restriction to the diet for 13 weeks, but scurvy symptoms were not apparent. When the volume of milk reached 75 cc. per individual daily the protection against scurvy over a period of 18 weeks was complete (Chart 7). These particular results are instructive. They indicate that the ration used for the production of this milk was

CHART 7. It required 75 cc. of dry feed milk daily per individual to protect against scurvy, while 30 to 50 cc. of summer pasture milk accomplished the same thing. This experiment shows that our dry feeds do contain some antiscorbutic, but not enough to protect a guinea pig when fed on the material directly.

not wholly devoid of the antiscorbutic vitamine. Although dried and at least a year old the materials fed these cows still had some antiscorbutic potency. This fact probably has very great importance in its relation to the antiscorbutic needs of species other than the guinea pig or man. It demonstrates that there is a measure of this vitamine in feeds dried as these were, but that a concentrating organ such as the mammary gland and a particularly sensitive animal such as the guinea pig are necessary for a demonstration of this fact. For the first 3 to 4 weeks on an allowance of 75 cc. of milk per individual not all of it was consumed, the consumption records showing from 55 to 65 cc.

per individual daily. After that time, however, the total 75 cc. were consumed.

With 100 cc. of dry feed milk daily per individual perfect protection against scurvy was afforded (Chart 8). These animals were well matured and their records are those of maintenance. For the first 10 weeks their consumption record varied from 87 to 95 cc. daily per individual. Thereafter the entire amount was consumed.

Manifestly then the diet may have a very pronounced influence upon the concentration of the antiscorbutic vitamine in the milk, being richer in this substance when the animals receive fresh

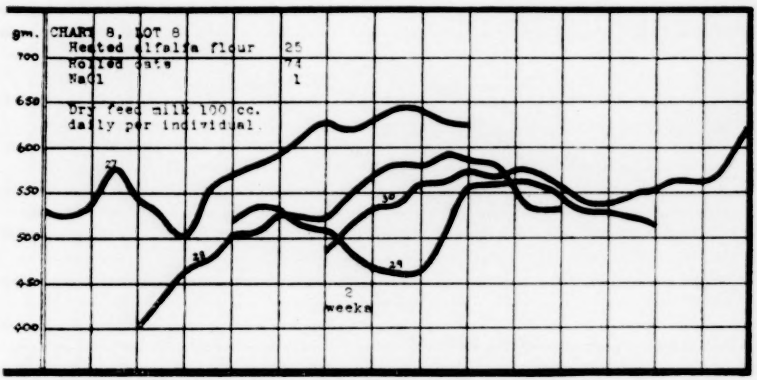


CHART 8. Showing the full protection against scurvy by 100 cc. of dry feed milk allowed daily per individual.

green materials in the diet or ration than when the ration is made up of air-dried materials. Apparently the mammary gland has no power to synthesize this vitamine and derives it preformed from the food. These experiments make it clear that in addition to the antineuritic vitamine the concentration of the antiscorbutic vitamine in milk is dependent upon its concentration in the ration.

We next investigated the antiscorbutic properties of milk made under normal winter feeding conditions. Winter feeding of dairy cows, at least in the corn belt, generally includes corn silage in the ration. Corn silage probably varies in its antiscorbutic properties. It is possible that silage made from fresh green corn is more potent in respect to its antiscorbutic content

than that made from corn killed by frost and withered and dried before it is placed in the silo. On this matter we have no data at present and will only present in this publication our experience with milk produced during the winter of 1920 from dried grains, hays, and a corn silage made from corn that had well matured and partly dried, but had not been frozen. It was put in the silo with the addition of water, a practice necessitated by its dryness. Whether processes of fermentation, involving particularly carbohydrate metabolism and acid production such as occur in silage making, are destructive to the antiscorbutic

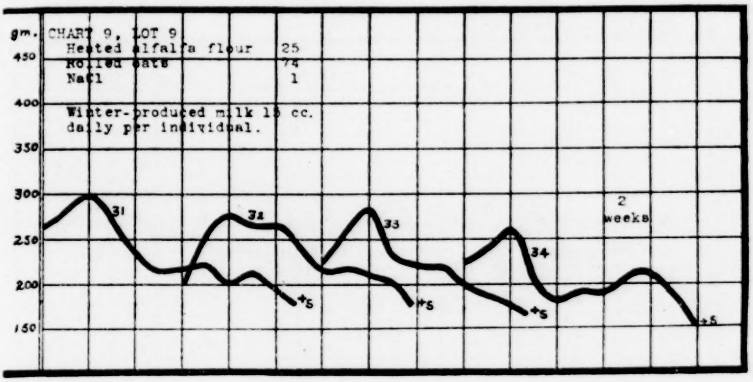


CHART 9. 15 cc. per individual daily of winter-produced milk afforded little protection against scurvy. This milk was from cows receiving a ration of dried grains and dried hays + silage, made from well matured and field-dried corn, but non-frosted.

vitamine is under investigation. We shall use the expression "winter-produced milk" as indicating a ration containing silage of the character described above.

The basal ration used for the guinea pigs was the same as in all other experiments and consisted of heated alfalfa hay, rolled oats, and common salt. The alfalfa was ground as a meal and mixed with the oats and salt in the proportion shown in the charts. With 15 cc. of winter-produced milk (Chart 9) all individuals succumbed to scurvy in 10 to 12 weeks. There was an initial gain in weight, but thereafter a gradual decline to death. These animals as a group lasted somewhat longer than the group

receiving 15 cc. daily per individual of dry feed milk which would indicate either that the individuals were more resistant or that the milk was slightly richer in antiscorbutic properties. However, its potency in the antiscorbutic was in a class with the dry feed milk and not with the green pasture milk. When 30 cc. of winter-produced milk were allowed per individual daily three of the group developed scurvy early, but prolonged their existence for 9 to 10 weeks (Chart 10).

One animal in this lot, No. 38, developed scurvy symptoms as early as the other members of the group, but lived much longer. There was a slow but gradual decline in weight by this individual

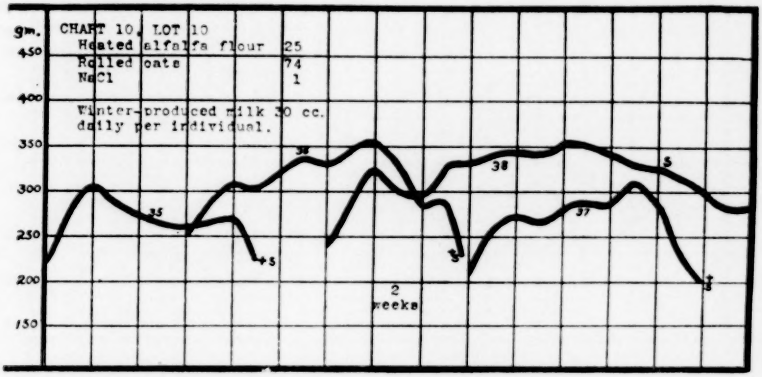


CHART 10. 30 cc. per individual daily of winter-produced milk (silage) was ineffective as an antiscorbutic when used with our basal ration of heated alfalfa flour and rolled oats.

as the scurvy symptoms became more aggravated, although it was not dead at the end of 18 weeks.

With 50 cc. or more of this milk daily per individual the protective action was more nearly complete (Chart 11). Three of this group appeared to be protected against scurvy for a period of 15 to 16 weeks. One of the group, No. 41, developed scurvy in the 11th week and died of the disease in the 14th week. These results should be contrasted with the records shown in Charts 3 and 6, respectively. With 50 cc. of summer pasture milk allowed daily per individual none of the group developed scurvy. With 50 cc. of dry feed milk three developed scurvy and one died from

some unknown cause. On 50 cc. of winter-produced milk one of the group developed scurvy. An allowance of 75 cc. daily per individual of winter-produced milk fully protected all the animals in Lot 12 against scurvy (Chart 12).

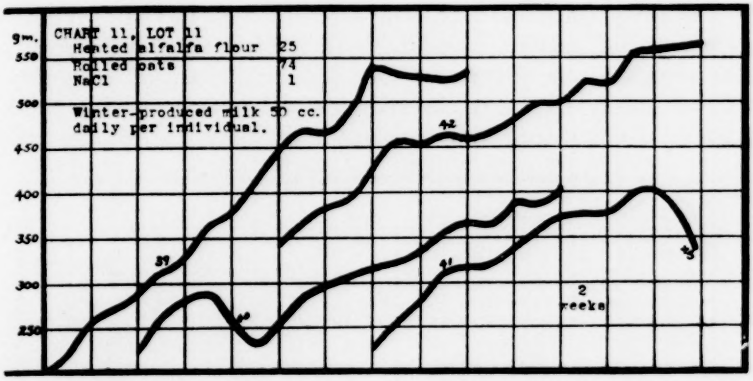


CHART 11. When 50 cc. of winter-produced milk were allowed daily per individual there was considerable protection against scurvy. Three of the group were fully protected for 16 weeks; one of the animals developed scurvy at the end of 11 weeks. Evidently this amount of milk was an insufficient source of the antiscorbutic vitamine for complete protection of all individuals.

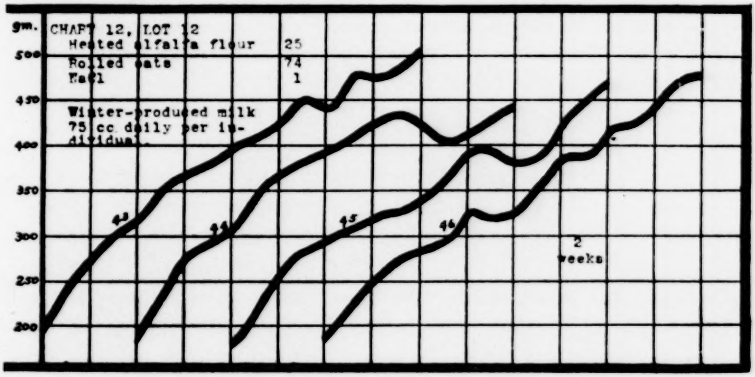


CHART 12. Showing the complete protective action against scurvy of 75 cc. daily per individual of winter-produced milk over a period of 14 weeks.

The winter feeding of dairy cows may very often include the roots of certain plants. Those most likely to be used in this country are such roots as the mangel, sugar mangel, or sugar beet. Carrots or parsnips may at times have limited use, but such roots as the rutabaga or turnip—the former reported by English investigators[8] to be comparatively rich in the antiscorbutic—are not likely to find a place in the feeding of dairy cows. This is due to the danger of producing milk tainted with the odor of sulfur oils. Our observations are limited to milk produced by a ration consisting of grains, mixed hay, a small amount

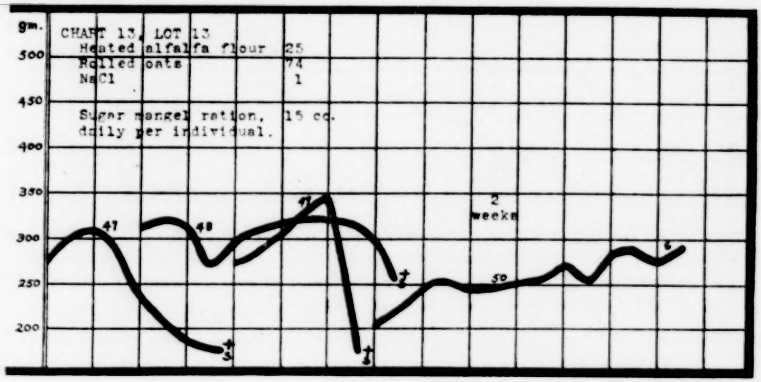


CHART 13. Showing the antiscorbutic properties of a milk produced from grain, dried hay, 10 pounds of silage, and 30 pounds of sliced sugar mangels daily. The silage was the same as used in all other experiments. 15 cc. of this milk per individual daily did not protect three of the animals in this group against scurvy. One animal was protected for 11 weeks, when initial symptoms of scurvy developed.

of silage (10 pounds per day), and the hybrid sugar mangel (*Beta vulgaris, var.*). This root is a cross between the mangel and sugar beet. About 30 pounds of the sliced beets were fed daily per individual. Chick and Rhodes have reported that the raw beet root—presumably the sugar beet—has relatively low antiscorbutic properties; they found, by the use of 20 cc. of the juice from this root, that protection to guinea pigs against the disease was negative, while 2.5 cc. of raw Swede juice (rutabaga) prevented the development of scurvy.

[8] Chick, H., and Rhodes, M., *Lancet*, 1918, ii, 774.

The sugar mangel milk was relatively low in antiscorbutic properties. 15 cc. of this milk added to our regular basal ration afforded little protection to growing guinea pigs (Chart 13). This protection was similar to that shown by the silage-produced milk. With 30 cc. of this milk allowed daily per individual (Chart 14) the onset of scurvy symptoms was not delayed in two of the cases, Nos. 51 and 52, but Nos. 53 and 54 were protected against scurvy for 11 weeks. Apparently the concentration of the antiscorbutic vitamine in the sugar mangel milk was not of such a magnitude as to prevent in all individuals the development of scurvy on our basal ration plus 30 cc. of this milk daily.

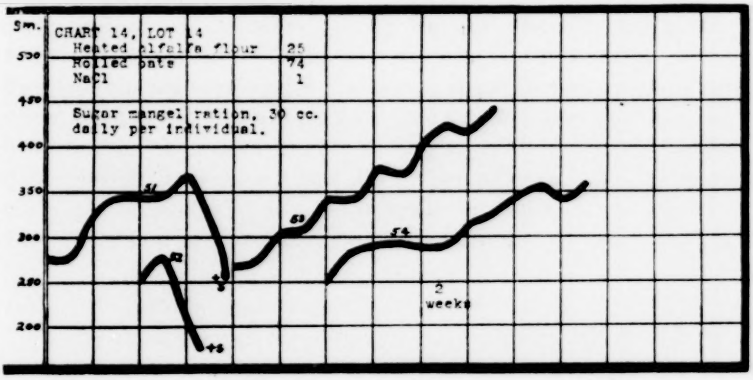


CHART 14. 30 cc. of sugar mangel milk daily per individual failed to protect two individuals in this lot. The other two animals were protected against scurvy by this quantity of milk for 11 weeks.

SUMMARY.

1. Evidence is presented showing that the concentration of the antiscorbutic vitamine in milk is dependent upon the diet.

2. Summer pasture milk is much richer in this nutritive factor than dry feed milk or winter-produced milk, involving the use in the ration of corn silage or sugar mangels.

3. 15 cc. daily of summer pasture milk imposed upon the basal ration used afforded protection against scurvy for 20 weeks to one guinea pig. However, for most individuals this quantity

of milk was insufficient for scurvy prevention. 50 cc. was a safer limit for complete protection.

4. At least 75 cc. daily per individual of dry feed milk were needed for complete protection against scurvy. The fact that milk produced from such dry feeds as used contained a measure of the antiscorbutic vitamine shows that the dry feeds themselves are not completely devoid of this nutritive factor. Their content in this vitamine is too low for protection of a guinea pig against scurvy, but the mammary gland, through a process of special selection and concentration in the milk produced, becomes an effective means of showing the presence of the antiscorbutic, especially in old dried feeds.

5. Silage made from corn well matured and partly dried before putting into the silo did not greatly enhance the concentration of the antiscorbutic factor in the milk. The same statement applies to the use of sugar mangels. Silage or sugar mangel milk was in a class with the dry feed milk and not of the order of green summer pasture milk in respect to its content of the antiscorbutic vitamine. The sugar mangel milk was a somewhat better source of this vitamine than the silage milk, but our data on this point are at present too limited to warrant an unqualified statement.

EXPLANATION OF PLATE 4.

FIG. 1. A normal guinea pig. 15 cc. daily of summer pasture milk protected against scurvy when the remainder of the ration consisted of heated alfalfa flour 25 parts, rolled oats 74 parts, and common salt 1 part. Photographed in the 12th week of restriction to this diet.

FIG. 2. A scorbutic guinea pig. 15 cc. daily of dry feed milk did not protect against scurvy when the remainder of the ration consisted of heated alfalfa flour 25 parts, rolled oats 74 parts, and common salt 1 part. Photographed after 4 weeks restriction to this diet.

FIG. 3. Showing early stages of scurvy. 15 cc. daily of winter-produced milk (silage + dry grain and dry hay) did not effectively protect against scurvy when the remainder of the ration consisted of heated alfalfa flour 25 parts, rolled oats 74 parts, and common salt 1 part. Photographed after 4 weeks restriction to the diet. The silage was made from field-dried, but non-frosted corn.

FIG. 1.

FIG. 2.

FIG. 3.

(Hart, Steenbock, and Ellis: Antiscorbutic potency of milk.)

HYDROGEN ION CHANGES IN THE MOSAIC DISEASE OF TOBACCO PLANTS AND THEIR RELATION TO CATALASE.

By R. B. HARVEY.

(From the Office of Plant Physiological and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture, Washington.)

(Received for publication, May 13, 1920.)

A number of determinations have been made upon the catalase[1] and oxidase[2] relations in the mosaic diseases. The author has confirmed the findings of previous authors that the catalase activity is decreased and the oxidase activity (Bunzell) increased in the mosaic areas. To account further for the abnormal growth in the infected areas and for the decrease in catalase, an attempt was made to determine the H^+ relations in the healthy and diseased tissues.

H^+ concentration determinations were made by the potentiometric method on freshly expressed juices. Tobacco leaves were collected from material of known heredity grown by Dr. Allard of the Division of Tobacco Investigations, at Arlington, Virginia. In all cases samples of 10 pounds or more of leaves were taken at the same time from healthy and diseased plants of the same variety growing in the same soil. The samples were ground in a meat chopper and expressed by hand through cheese-cloth, or a portion was taken from the middle of each leaf and treated as above. In every case each sample was made up of old and young leaves from four or more plants. It was found that tobacco leaf juice could be diluted somewhat without changing the H^+ concentration. Hence it was expected that minor differences in

[1] Chapman, G. H., *Massachusetts Agric. Exp. Station, Bull. 175*, 1917.

[2] Woods, A. F., *Science*, 1900, xi, 17. Bunzel, H. H., *U. S. Dept. Agric., Bureau Plant Industry, Bull. 277*, 1913, 7; *J. Agric. Research*, 1914, ii, 373; *Biochem. Z.*, 1913, l, 185.

concentration due to the method of expression would not affect the H^+ concentration of the juice greatly. By using the same method for all samples the results should be comparable. Better methods are desirable but not available. The permissible dilution is not sufficiently great to allow a colorimetric method to be used for determining the H^+ concentration on the dark-colored juice. The concentration of buffer is low and for this reason the expressed sap is subject to considerable change of H^+ concentration during protein precipitation. For this reason it is necessary to work rapidly with such juices and a colorimetric method would be desirable. Freezing of the tissue was not permissible.

In Table I are given the changes in pH at various dilutions on the same sample of juice.

TABLE I.

Dilution of juice.	pH
Undiluted.	5.753
10 : 1	5.755
5 : 1	5.788
1 : 1	5.849

A number of tests were made at different times and in most cases the mosaic plants show an H^+ concentration somewhat greater than healthy plants of the same variety. In some cases the mosaic and healthy plants show approximately the same acidity. In no case was the H^+ concentration of healthy plants greater than that of mosaic plants within the limit of error.

Catalase determinations were made in the Van Slyke apparatus for the determination of amino-acids in a constant temperature room at 30°C. 10 gm. of fresh leaf were cut up with a knife and ground in a mortar with quartz and sufficient sodium phosphate buffer mixture (C_{H^+} 2.7×10^{-8}) or $CaCO_3$ to establish the H^+ concentration at a desired point. All the samples were made of portions of a large number of leaves. Dilutions of 10 gm. of leaves (green weight) to 250 cc. were found to be of a convenient concentration.

In general the catalase determinations show a lesser difference in the activity of mosaic and healthy plants than those made by Chapman. This is probably for the reason that Chapman did

not regulate the H^+ concentration in his experiments. If the H^+ concentration is not established at about $C_H{}^+ 10^{-8}$, one has the combined effect of low catalase content in the mosaic plants, lesser activity of this catalase at the greater H^+ concentration of mosaic plants, and a high rate of destruction of the catalase originally present.

In Table II are given the H^+ concentration and catalase activity of a number of samples. In those cases in which the mosaic plants show an H^+ concentration considerably greater than the

TABLE II.

Hydrogen Ion Concentration and Catalase Activity of Mosaic and Healthy Tobacco Leaves.

Healthy.			Mosaic.		
pH	$C_H{}^+ \times 10^{-8}$	Average O_2.	pH	$C_H{}^+ \times 10^{-8}$	Average O_2.
		cc.			cc.
5.497	7.016	17.6	5.247	8.768	17.2
5.330	8.261	10.5	5.327	8.280	6.9
5.430	7.559	13.7	5.064	9.713	7.5
5.247	8.768	13.5	5.268	8.645	13.2
5.280	8.573	13.6	5.180	9.149	11.7
5.474	7.210		5.335	8.228	
5.403	7.760		5.297	8.470	
		19.7			17.6
		16.3			11.7
		15.3			10.7
Average.					
5.380	7.878	15.0	5.245	8.750	12.1

healthy plants, the catalase activity in the same lot of leaves is correspondingly low. The presence of the virus in the mosaic-diseased areas produces a condition in which abnormal metabolism and growth are associated with hydrogen ion changes and low catalase activity.

As in the case of acidosis of animals, a great change of H^+ concentration is not likely to be found for it would result in death of the tissue. The increase in H^+ concentration and decrease in catalase activity in the living diseased areas can only be small if these factors are important in the life of the cells.

SUMMARY.

The H^+ concentration of juice expressed from mosaic tobacco leaves was found to be somewhat greater than that from healthy plants, the average for mosaic plants being C_{H^+} 8.750×10^{-6} and for healthy plants C_{H^+} 7.878×10^{-6}.

Catalase activity in the mosaic leaves is decreased with increasing hydrogen ion concentration.

EVIDENCE INDICATING A SYNTHESIS OF CHOLESTEROL BY INFANTS.

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(Received for publication, May 25, 1920.)

Various foodstuffs derived from plants contain plant sterols which are closely similar to cholesterol. Fairly satisfactory evidence of the availability of these plant sterols as a source of cholesterol in the animal organism has been obtained. Fraser and Gardner[1] found that phytosterols fed to rabbits on a diet of extracted bran cause an increase in the antihemolytic power of the blood serum to the same extent as when cholesterol is added to this diet. They found also[2] that when a diet of extracted bran is followed by a bran diet from which phytosterols have not been removed there results an increase in the cholesterol content of the blood. It is therefore conceivable that cholesterol, which is so prominent a structural constituent of animal tissues, may be derived altogether from cholesterol and phytosterols present in food. The complex chemical character of cholesterol, and evidence that it is conserved within the organism by being to some extent reutilized after excretion in the bile, are points in favor of this inference.

Most of the experimental evidence at hand suggests an inability of the organism to synthesize cholesterol. Ellis and Gardner[3] from analyses of eggs and of newly hatched chicks found the same quantity of cholesterol in chicks as was found to be stored

[1] Fraser, M. T., and Gardner, J. A., *Proc. Roy. Soc. London, Series B*, 1909, lxxxi, 230.

[2] Fraser, M. T., and Gardner, J. A., *Proc. Roy. Soc. London, Series B*, 1909–10, lxxxii, 559.

[3] Ellis, G. W., and Gardner, J. A., *Proc. Roy. Soc. London, Series B*, 1909, lxxxi, 129.

in unincubated eggs. Gardner and Lander[4] found no evidence of cholesterol synthesis in chickens fed an extracted food for several weeks after hatching. Their control chickens, however, fed on an ordinary diet, did not add to their cholesterol content during the same period. Evidence of a cholesterol intake requirement, at least during this early period of the chickens' development, is not provided by the results of this experiment. Ellis and Gardner[5] state that the amount of coprosterol in the feces of dogs and cats fed a diet of lean meat or sheep brains can always be more than accounted for by the estimated quantity of cholesterol in the food. The same investigators,[6] from determinations of the coprosterol content of stools from an adult subject who received several high protein- and fat-containing diets, are inclined to regard the cholesterol intake as equivalent to the coprosterol excretion. In these "balance" experiments the coprosterol was obtained from the non-saponifiable fraction of stool extracts by crystallizing it out several times in diluted alcohol. Coprosterol forms crystals sluggishly and it is not easy to be sure that a quantitative yield has been obtained. It is permissible to doubt whether all the coprosterol present in stool samples can in this way be collected for weighing. Moreover, the cholesterol content of the diets used by Ellis and Gardner was not directly determined, but was estimated from rather scant existing data. The balance which they found in favor of cholesterol intake cannot, therefore, be regarded as more than roughly indicated.

Dezani[7] has reported that white mice fed on extracted diets over a period of several weeks not only do not lose cholesterol but actually add considerably to their cholesterol content. Analyses of six controls at the beginning of the experiment gave an average cholesterol content of 0.158 gm., whereas the average amount of cholesterol found in sixteen mice after 3 weeks on various extracted diets was 0.242 gm. The author concludes

[4] Gardner, J. A., and Lander, P. E., *Proc. Roy. Soc. London, Series B*, 1913–14, lxxxvii, 229.

[5] Ellis, G. W., and Gardner, J. A., *Proc. Roy. Soc. London, Series B*, 1909, lxxxi, 505.

[6] Ellis, G. W., and Gardner, J. A., *Proc. Roy. Soc. London, Series B*, 1912–13, lxxxvi, 13.

[7] Dezani, S., *Arch. farm.*, 1914, xvii, 4.

from the result that growing white mice readily synthesize cholesterol. If the assumption that the extracted diets were thoroughly free of cholesterol and phytosterols is correct, it is difficult to escape this inference. It should be mentioned that none of the diets used was adequate for continued growth and maintenance of the animals. After 2 weeks they became unhealthy and began to lose weight. As evidence that healthy mice regularly synthesize cholesterol, the result of this experiment cannot be accepted without reservation. The construction of a suitable diet for this plan of experiment, *i.e.* a diet quite free of cholesterol and phytosterols, but adequate in all other respects for growth and maintenance, presents considerable difficulties and has not yet been successfully undertaken.

In the experiments to be reported in this paper the relation of the preformed cholesterol in the food to the total cholesterol metabolism was studied in infants by comparing carefully the intake and excretion of cholesterol. The suggestion to undertake this simple balance experiment was obtained from the observation of Müller[8] that cholesterol in the case of infants, or of adults on a continued milk diet, is not changed to coprosterol in the intestine. This fact and the probability that the methods at hand for determining cholesterol in the blood could be successfully applied to stool and milk samples seemed to offer opportunity for an accurate measurement of cholesterol taken in with the food and that excreted in the feces.

It was assumed that the finding of a regularly positive balance in favor of cholesterol intake in the case of normal infants who were gaining in weight on usual diets could be taken as evidence that the cholesterol of the tissues is probably altogether derived from the preformed cholesterol in the food, whereas a regularly negative balance under the same conditions would quite definitely indicate a synthesis of cholesterol within the organism.

[8] Müller, P., *Z. physiol. Chem.*, 1900, xxix, 129.

Method of Determining Cholesterol.

Denis and Minot[9] have quite thoroughly studied the cholesterol content of milk, using the method devised by Bloor[10] for determination of cholesterol in the blood. In our hands this simple and direct method proved to be unreliable for accurate measurement of cholesterol in stool and milk samples. The color obtained was usually found to differ so much in quality from that of the standard that only an approximate comparison was possible. In the case of milk samples so much fat was taken out by the hot chloroform as to interfere subsequently with the transparency of the reading solution. A preliminary saponification and removal of the soaps in order to bring the cholesterol in cleaner fashion into the reading solution seemed indicated. After considerable experimenting a modification of the Autenreith and Funk[11] method was devised, by means of which the cholesterol could be obtained in an entirely satisfactory condition, and without the expenditure of the time and patience required by the original method. The alteration consisted in applying Kumagawa and Suto's[12] plan of using diluted alcohol and petroleum ether as a means of separating non-saponifiable material from soaps.

The method as applied to milk was carried out as follows.

10 cc. of milk were digested in a beaker with 20 cc. of 30 per cent KOH for 2 hours on a steam bath. The digest, which then amounted in volume to about 15 cc., was transferred with small rinsings of water to a 100 cc. graduated cylinder. 25 cc. of 95 per cent alcohol and about 60 cc. of petroleum ether were then added, and the cylinder was shaken for 5 minutes. The petroleum ether, which separated quickly and sharply from the diluted alcohol, was then blown over through capillary tubing into a separatory funnel and a fresh quantity of petroleum placed in the cylinder. After shaking the cylinder again for 5 minutes the ether was transferred to the separatory funnel. The ether in the separatory funnel was then washed with about 20 cc. of water, transferred to a beaker, and evaporated on the steam bath. The dry residue was then dissolved and transferred to a small graduated cylinder by means of small portions of hot chloroform. The color was developed and the reading made in the colorimeter in the usual way. As

[9] Denis, W., and Minot, A. S., *J. Biol. Chem.*, 1918, xxxvi, 59.

[10] Bloor, W. R., *J. Biol. Chem.*, 1916, xxiv, 227.

[11] Autenreith, W., and Funk, A., *Münch. med. Woch.*, 1913, lx, 1243.

[12] Kumagawa, M., and Suto, K., *Biochem. Z.*, 1908, viii, 315.

tested by adding known amounts of cholesterol to the aqueous digest this plan of separating the cholesterol by shaking out twice with diluted alcohol and ether was found to give results which are practically theoretical, inasmuch as the small amount of cholesterol left behind cannot be measured by the colorimeter. Moreover, the formation of an emulsion which nearly always interferes with the use of the Autenreith and Funk method for milk samples rarely takes place. In determining cholesterol in the stools the plan used was to extract a large sample of dried stool with boiling alcohol and ether (3:1) for 1 hour, with a reflux condenser, and then to saponify an aliquot of the extract. The cleanliness with which the method used separates the non-saponifiable material is indicated by the fact that the non-saponifiable fraction from stools which is practically altogether cholesterol (see below) appears on the bottom of the beaker after evaporation of the petroleum ether as wide transparent plates exactly resembling the crystalline residue obtained by evaporating an ethereal solution of pure cholesterol.

A few comparisons with results obtained by the digitonin method of Windaus[13] were made. This method is not easily applicable to stool and milk samples. In the case of milk, 25 gm. of Merrell-Soule whole milk powder were first extracted with alcohol and ether. The extract was then saponified with sodium ethylate, dried with a quantity of sodium chloride, and extracted with ether in a Soxhlet.[14] After the ether had been evaporated, the residue was taken up in alcohol and the cholesterol precipitated with digitonin. A single determination carried out in this way gave a measurement of 0.78 mg. of cholesterol per gm. of dried milk. As determined by the colorimetric method the cholesterol content was 0.80 mg. per gm. The cholesterol content of two samples of dried stools measured by the digitonin method was 19.3 and 9.1 mg. per gm. as compared with 19.9 and 10.0 mg. per gm. by the colorimetric method. Taking into consideration the amount of preliminary manipulation which is necessary to prepare the samples for the digitonin method an agreement between the two methods within 10 per cent may be regarded as fairly satisfactory. A more thorough comparison of results obtained by the two methods was not possible because of lack of digitonin.

[13] Windaus, A., *Z. physiol. Chem.*, 1910, lxv, 110.
[14] Ritter, E., *Z. physiol. Chem.*, 1901–02, xxxiv, 430.

Subjects and Plan of Experiments.

The subjects used were four infants taken from the hospital wards several weeks after admission for correction of nutritional disturbances, and at a time when they were gaining steadily and satisfactorily in weight.

They were comfortably immobilized on a metabolism frame for a period of 3 days. The cholesterol contained in the milk taken and in the stools passed during the period was determined by the method described above.

In order to obtain a stool collection corresponding as closely as possible to the food intake, a small amount of charcoal was placed in the first and in the last feeding of the experimental period. The food given was a milk or milk modification on which the infant had gained steadily during a fore period of from 3 to 10 days. The quantity of cholesterol present in the food was not less than is contained in the modifications of milk which are usually given to normal infants at ages corresponding to those of the subjects used for these experiments. In all except one instance the infants continued to gain during the experimental period. The food was always well taken and without evidence of disturbed gastrointestinal function, except that in the instance of Subject R while receiving whole milk there was quite marked constipation. The stools from the other infants were normal in character and frequency. It must be said that the body weights of all the subjects of this experiment were much below the average which obtains for healthy infants of corresponding ages. The extent to which they fall short of this measure of a normal state of nutrition is indicated in the tables. The assumption that at the time of experiment their nutritional process was proceeding normally is based on the fact that they were gaining gradually in weight without discernible symptoms of nutritional disturbance. It must be admitted that this premise cannot be used without reservation.

Results of Experiments.

In order to test the statement of Müller that cholesterol is excreted unchanged in infants' stools, a quantity of non-saponifiable material sufficiently large for weighing was obtained from

each of the stool specimens by the method described above, except that in this case a large sample of the alcohol-ether extract was taken and after saponification the non-saponifiable material shaken out with appropriately large volumes of diluted alcohol and petroleum ether. But a single extraction with petroleum ether was made as a quantitative separation was not attempted. The petroleum ether was washed several times with distilled water, then evaporated, and the weight of the residue determined. The residue was then dissolved in chloroform and its cholesterol content measured colorimetrically. By this procedure it was found that the cholesterol in the stools from all the experimental periods was approximately equivalent to the total non-saponifiable material present (Table I). Apparently neither coprosterol

TABLE I.

Subject.	Samples of non-saponifiable material.	Cholesterol content determined colorimetrically.
	mg.	*mg.*
O'D.	10.7	11.5
S.	12.3	13.0
R.	11.6	10.8
C. 1	10.2	9.8
C. 2	14.0	13.1

nor other non-saponifiable substance was present in appreciable amounts in these stools. In two instances the melting point of the non-saponifiable material was compared with that of pure cholesterol and was found to be the same (147–148°C.). The melting point for coprosterol is given as 95–96°C. This result indicates that in all probability the cholesterol content of infants' stools is equivalent to the total cholesterol excretion.

The relation of cholesterol intake to cholesterol excretion is shown in Table II. It will be seen that in all the experimental periods more cholesterol is found in the stools than was contained in the food. Moreover, the discrepancy is large, the excretion of cholesterol being 1.7 to 3.4 times the intake.

TABLE II.

Subject.	Age.	Weight.	Average normal weight.	Food per day.	Gain in weight per day during experimental period.	Cholesterol in food per day.	Cholesterol in stools per day.	Ratio cholesterol excretion: cholesterol intake.
	mos.	*kg.*	*kg.*		*gm.*	*gm.*	*gm.*	
O'D.	3	4.1	5.7	360 cc. milk. 360 " barley water. 36 gm. cane sugar.	70	0.050	0.114	2.3
S.	4	5.1	6.3	540 cc. milk. 360 " water. 45 gm. cane sugar.	25	0.072	0.212	2.9
C. 1	4½	4.9	6.6	675 cc. woman's milk.	20	0.107	0.177	1.7
C. 2	5	4.9	6.8	540 cc. milk. 360 " water. 45 gm. cane sugar.	0*	0.070	0.125	1.8
R.	19	6.2	10.9	1,200 cc. whole milk.	10	0.125	0.424	3.4

* Weight stationary.

DISCUSSION.

The fact that cholesterol excretion was found to be regularly greater than cholesterol intake in the case of several infants who were gaining in weight and receiving a usual amount of cholesterol in their food can be interpreted as evidence that cholesterol is to some extent synthesized within the organism. The large discrepancy between intake and excretion lends strength to this inference. The necessary premise that the subjects of these experiments were in an altogether normal state of nutrition unfortunately cannot be warranted, as they all were at the time convalescing from more or less serious nutritional disturbances. It may be said, however, that subjects O'D and S were as apparently vigorous and healthy infants as can usually be found in hospital wards. If cholesterol metabolism is entirely dependent on an intake of preformed cholesterol in the food, it is very difficult to understand how the subjects of these experiments managed to gain in weight and present no symptoms of disturbed nutritional processes while exhibiting a large negative cholesterol balance.

The value of the results of these experiments and of any inference which can be drawn from them depends largely on the degree of accuracy with which the cholesterol content of the milk samples was measured. Milk contains about one-tenth as much cholesterol as blood and a relatively very large amount of fat. It was in our experience not possible to make an accurate colorimetric measurement of the cholesterol without first separating it from the milk fat. We believe that a closely quantitative separation is obtained by the method described. Analyses of ten samples of cow's milk by this method gave an average result of 13.0 mg. per 100 cc., the figures ranging from 9.8 to 15.5 mg. per 100 cc. Denis and Minot using Bloor's method found an average value of 14.5 mg. per 100 cc. In the fifteen samples analyzed the cholesterol content was between 10.5 and 17.6 mg. per 100 cc. Apparently Bloor's direct method applied to milk will usually give a value for cholesterol content which is 10 to 30 per cent higher than would be obtained by the saponification method used in these experiments. The discrepancy found between the intake and excretion of cholesterol by these infants was, however, so large that, even if it is assumed that Bloor's method measures the cholesterol content correctly and the method used is debited with a minus error of 30 per cent, the fact of a large negative balance would not be altered.

SUMMARY AND CONCLUSIONS.

Analyses of the non-saponifiable fraction of infants' stools confirms the observation of Müller that cholesterol is excreted in infants' stools without undergoing to appreciable extent change to coprosterol.

Making use of this fact we determined the cholesterol intake and excretion of four infants over periods of 3 days. The subjects used were gaining in weight and were receiving a usual amount of cholesterol in their food. In all the experimental periods a much larger excretion than intake was found, the quantity of cholesterol in the stools being 1.7 to 3.4 times that which was present in the food. This result is interpreted as indicating a synthesis of cholesterol within the organism.

A method for determining cholesterol in milk and stool samples is described.

DEGREE OF ALKALINITY NECESSARY FOR THE PHLOROGLUCIN TEST FOR FORMALDEHYDE.

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The successful use of phloroglucin as a reagent for the detection of free formaldehyde depends on the recognition of two important factors; namely, purity of the reagent and degree of alkalinity. As to purity, the white crystalline variety (Merck's reagent) of phloroglucin only should be used and not the brown amorphous variety, which greatly modifies the characteristic red color with formaldehyde.

The degree of alkalinity necessary for a positive reaction with formaldehyde will be appreciated from the following observations which were made with ordinary buffer mixtures of primary and secondary phosphates, sodium bicarbonate, sodium carbonate, and sodium hydroxide of known hydrogen ion concentrations. 0.5 cc. of 1 per cent phloroglucin in alcohol was added to 10 cc. of the solutions representing the different degrees of alkalinity and containing 1:10,000 parts of absolute formaldehyde in one set of experiments and 1:100 parts of formaldehyde in another set. The results with the two different concentrations of formaldehyde were identical and are summarized in Table I.

From this it is seen that the degree of alkalinity necessary for a minimal positive formaldehyde reaction is rather high, namely pH 12.13, or the equivalent of the alkalinity of about 0.01 N solution of sodium hydroxide, or about 0.04 per cent. An optimal positive reaction requires 0.1 N hydroxide or carbonate (pH of about 13.0). The reaction cannot occur in solutions of bicarbonate since this salt cannot provide a high enough degree of alkalinity. Ammonia water and very weak solutions of sodium hydroxide also are inadequate. Lack of attention to this

detail, *i.e.* the proper degree of akalinity, probably explains the unsuccessful use of the reagent in studies on formaldehyde liberation from hexamethylenetetramine by some investigators. The practice of using the phloroglucin in alcoholic solution followed by the haphazard addition of alkali is unsatisfactory because under these conditions the proper adjustment of the degree of alkalinity may be easily overlooked. Mere bluing of red litmus paper may not be sufficient. More reliable results are obtained by the use of a reagent, consisting of 1 per cent phloroglucin in 10 to 20 per cent sodium hydroxide. This keeps almost indefinitely and insures the required degree of alkalinity for all ordinary purposes, including the application of the reagent for the detection of formaldehyde in urines and other body fluids, except bile and blood, containing hexamethylenetetramine.

TABLE I.

Solution used.	Degree of alkalinity.	Formaldehyde reaction.
	pH	
0.1 N $NaHCO_3$.	About 8.2.	Negative (yellow color).
Phosphate mixture.	8.4	" " "
0.001 N NaOH.	12.12	" (yellowish color).
0.01 N NaOH.	12.13	Positive (reddish).
0.1 N NaOH.	13.07	" (deep red).
0.1 N Na_2CO_3.	About 13.00.	" " "
1 N NaOH.	14.05	" " "

Trendelenburg[1] makes use of a reagent containing 1 per cent phloroglucin in 33 per cent sodium hydroxide. The violet color which the reagent assumes if made with weaker alkali, according to him, does not occur under these conditions. After several years experience with the reagent containing 10 to 20 per cent alkali, I have found that the violet color, which develops while the phloroglucin is undergoing solution in the hydroxide, disappears on standing, leaving the reagent almost colorless, or at most yellowish. Furthermore, the violet color does not at all interfere with the development of the positive formaldehyde reaction even in extremely weak and quantitative solutions, since this depends on a brilliant red color in high and orange reddish in weak concentrations.

[1] Trendelenburg, P., *Biochem. Z.*, 1919, xcv, 146.

In fact the gradations between the colors obtained with different concentrations of formaldehyde are fine enough for quantitative purposes. Such a method with the use of an artificial standard has been previously described by Collins and Hanzlik.[2] The same method can be used for the quantitative estimation of hexamethylentetramine.

CONCLUSIONS.

The phloroglucin test for free formaldehyde requires a rather high degree of alkalinity, *i.e.* pH 12.13, or the equivalent of the alkalinity of a 0.01 N sodium hydroxide solution, or about 0.04 per cent, for a minimal positive reaction, and 0.1 N hydroxide or carbonate for an optimal reaction (pH 13.0). The neglect of this important factor, and also the use of impure phloroglucin, may explain the unsuccessful use of the reagent by some. Used properly this is probably the most sensitive reagent for free formaldehyde known.

[2] Collins, R. J., and Hanzlik, P. J., *J. Biol. Chem.*, 1916, xxv, 231.

EXPERIMENTS ON CARBOHYDRATE METABOLISM AND DIABETES.

I. INTRAVENOUS GLUCOSE TOLERANCE OF DOGS.

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The present paper forms one of a series concerning the internal pancreatic function. Experiments dealing with quantitative relations between this function and the body mass and metabolism have already been in part presented elsewhere.[1] The series in this *Journal* will concern the nature of the pancreatic function and its rôle in metabolism. This series is fragmentary. The study was begun in the early period of the diabetic work at The Rockefeller Institute, as an attempt either to establish or disprove certain conceptions of normal and diabetic metabolism which had grown out of the previous investigation at the Harvard Medical School. Events compelled dropping this undertaking, and only a few experiments were worked in subsequently as time permitted. The portion completed may serve to clear the ground a little and reveal the scope and purpose of the original plan.

The present paper deals with the assimilation of intravenously injected glucose by dogs. Merck's anhydrous glucose was used throughout, and the concentration of solutions determined by simple weighing. Analyses for sugar in blood or plasma were performed by the original Lewis-Benedict[2] method, and those for urinary sugar by titration with Benedict's copper solution. Except when otherwise stated, the dogs were on a diet of bread and soup *ad libitum*, and experiments were begun 20 to 24 hours after

[1] Allen, F. M., *J. Exp. Med.*, 1920, xxxi, Experimental studies on diabetes, Series I, 363, 381, 555, 575, 587; xxxii, Series II (in press).

[2] Lewis, R. C., and Benedict, S. R., *J. Biol. Chem.*, 1915, xx, 61.

the last feeding. The method of injection requires special mention.

Interrupted injections were used, essentially according to the method of Blumenthal,[3] for two reasons; first, the experiments were mostly performed before Woodyatt had devised his excellent apparatus for uniform continuous infusions; second, one important purpose of the test was the comparison of the normal assimilation with that attained during exercise, and an interrupted method seemed indispensable for this purpose. Obviously, when short injections are given 15 or 20 minutes apart, there is a sudden flood of hyperglycemia, followed by a gradual decline to the time of the next injection. The excess passes rapidly into the tissues, and there is a certain basis for Blumenthal's conception of a "saturation limit" of the organism, in that it will hold a certain quantity of sugar like a sponge, and with identical dosage the assimilation is practically the same whether the injections are given continuously or at short intervals. It is true that Wilder and Sansum[4] criticized the irregularities of tolerance found in rabbits by different workers with the Blumenthal method, but there is no evidence that these irregularities were due to anything else than the failure of the earlier workers to take the precautions against fright, pain, and shock concerning which Woodyatt and his collaborators were so careful, and Woodyatt[5] recognizes that the results with his method and the Blumenthal method are essentially the same. At any rate, the plan of delivering fixed doses of glucose into a vein and allowing fixed intervals for its disposal is one valid method of testing the assimilative power. A few experiments with continuous infusion were also performed as controls.

The detailed procedure was as follows. The dogs used were sturdy, phlegmatic animals, thoroughly accustomed to the laboratory and to staying on the table without jumping down, and with all fear or nervousness lost through previous experience. The dietary tolerance of the diabetic ones was accurately known from long observations. An hour or so before the experiment, an area of the neck was shaved, if necessary, and a short portion of an

[3] Blumenthal, F., *Beitr. chem. Physiol. u. Path.*, 1905, vi, 329.

[4] Wilder, R. M., and Sansum, W. D., *Arch. Int. Med.*, 1917, xix, 311–334.

[5] Woodyatt, R. T., *The Harvey Lectures*, 1915–16, xi, 326.

external jugular vein exposed, with local anesthesia. The essential point is to clean a tiny portion of vein wall of every trace of fascia. The skin falls together so that the uncovered area is kept naturally moist. The dog is not tied or confined at any stage of the experiment. At the time for beginning, an assistant raises the dog's head. The operator takes a Luer syringe containing a few crystals of potassium oxalate and equipped with a needle, of No. 21 or 22 size and $\frac{3}{4}$ inch length. He draws blood into the syringe, then detaches the needle without removing it from the vein, and immediately attaches another syringe previously filled with the solution at proper temperature and makes the injection at a convenient rate, requiring perhaps 2 to 4 minutes according to the dose. There is no bleeding when the needle is withdrawn. The dog sits or lies quietly without restraint, generally dozing, till the time of the next injection. The needle is then inserted through the same hole in the vein wall as before, the blood sample drawn, and the injection made as before. The same hole in the vein is generally used throughout the whole experiment. The blood sugar values thus obtained are a minimum, because taken at the end of the interval. A few analyses have been made immediately after injection. In some experiments blood samples were taken only hourly. The method serves satisfactorily for comparisons of assimilation, but affords no exact reckoning of relations between the sugar levels in blood and urine.

Continuous injections were given through a small cannula tied into an external saphenous vein, while blood samples were taken from the external jugular. Here also the dogs were merely watched and not confined; they mostly dozed without noticing the cannula, but were free to rise and stretch as suited their comfort. The glucose solution was merely run in from a burette fixed at a suitable height. This generally held the quantity to be given in 15 minutes, and it was one person's duty to keep the flow uniform and in particular to make sure that one-third of the quantity was delivered every 5 minutes.

With both kinds of injections, the dogs were catheterized hourly or sometimes at shorter intervals. Permanent catheterization was not used. The glucose solutions were made up in distilled water except when saline is specified.

REMARKS AND CONCLUSIONS.

1. Agreement of Results.—As shown in Table XXXII, the results in normal dogs agree satisfactorily both among themselves and with Woodyatt's findings. Giving 1.8 to 2 gm. per kilo per hour, Sansum and Woodyatt[6] found ordinarily about 2 per cent of the dose excreted, with variations between individual animals reaching as high as 10 per cent of the dose in one animal. A similar close agreement is seen here between the dogs receiving 1.5 gm. per kilo per hour. Also in harmony with Woodyatt, the dosage of 1 gm. per kilo per hour is found to be slightly above the tolerance, with the single exception shown in Table IV, Period A. Variations in the concentration have, as shown by Woodyatt, no perceptible effect upon the assimilation. Even the different modes of administration, whether continuously or in four, three, or even two injections per hour, are apparently of minor importance, though this is not certain, and it is desirable that comparisons should be based upon the same method, as has been done in this series.

2. Demonstration of Alterations of Tolerance.—(A) A reduction of tolerance, as established by Wilder and Sansum[4] in human patients, is demonstrable in a broad sense in diabetic animals. Tables XXXII and XXXIII show that in general the excretion of sugar in partially depancreatized dogs is greater than that in normal dogs, and that this excretion increases with the severity of the diabetes. Also Tables XXIX, XXX, and XXXI show increase of the sugar excretion with aggravation of the diabetes in the same animal, and the latter two were thus used[7] to demonstrate the downward progress of diabetes on excessive diets of either carbohydrate or protein even in absence of glycosuria.

(B) This general rule is subject to very marked exceptions. A mildly diabetic animal (Table XX) remained free from glycosuria with dosage of 1 gm. of glucose per kilo per hour, the tolerance thus apparently surpassing that of normal animals. The difference between Periods A and E in Table XVII evidently indicates aggravation of the diabetes during the experimental period, but even at the close the diabetes was still very mild, so

[6] Sansum, W. D., and Woodyatt, R. T., *J. Biol. Chem.*, 1917, xxx, 155–173.
[7] Allen, F. M., *J. Exp. Med.*, 1920, xxxi, 567, 569.

that the dog was able to live on bread without glycosuria. Yet the excretion of 37 per cent of the dose by this animal was higher than that of any of the other dogs of the series, some of which were so severely diabetic that they were kept alive only on low protein-fat diets and would have gone into a hopeless condition if this tolerance had been overstepped for only a few days. The dog in Table XXVIII had one of the severest grades of diabetes in the series, which was thoroughly controlled by diet and reduction of weight. It was proper that the test should show the benefit of such thoroughness, but not that the assimilation should appear superior to that of the two non-diabetic dogs (Tables XV and XVI) and of nearly all the mildly diabetic ones. Other comparisons between diabetic dogs in Table XXXIII show that the sugar excretion is seldom an accurate measure of the degree of diabetes as known from the food tolerance and general clinical condition. The same applies to the gradations of assimilation in conditions short of diabetes. Thus the excretion is practically identical in Tables II and XV, though one animal was normal and the other was depancreatized to an extent involving a very great lowering of tolerance as demonstrable by alimentary and subcutaneous tests.[1] The animal in Table XVI, which was very close to diabetes, excreted a bare trifle more glucose than the normal dog in Table VI and actually less than that in Table VIII. Tests of assimilation based upon the rate of excretion in comparison with the rate of intravenous injection of glucose are therefore subject to wide errors.

(C) Determinations of the concentration of sugar in the blood are necessary for the correct interpretation of such experiments.

(*a*) This is the only means by which the important factor of the renal function can be judged. The latter is the outstanding cause of the apparent irregularities of tolerance in these experiments. The normal dog in Table IV showed no more than a doubtful reaction in the urine when the blood sugar in Period A was found as high as 0.173 per cent. It is self-evident that these analyses taken just before injections represent minimal values. This fact has occasionally been demonstrated by taking samples from the opposite jugular or a femoral vein as quickly as possible after finishing an injection; for example, a value of 0.457 per cent was thus found in Table III, and one of 0.770 per cent in the

diabetic dog in Table XXX, and 0.294 per cent about 2 minutes after an injection in Table XIX, Period A. This flood of sugar rapidly passes from the blood into the tissues, as already mentioned. It is obvious that the normal dog in Table IV had an exceptionally high renal threshold for sugar in Period A, especially as the temporary excess of sugar was necessarily greater with only two injections per hour; also in Period B the sugar elimination was relatively small compared with the hyperglycemia. This renal impermeability will readily explain the exceptionally high tolerance. The mildly diabetic dog in Table XVII had an exceptionally active excretory function for sugar. After discontinuance of injections in Period A, this dog had glycosuria with plasma sugar concentrations from 0.084 to 0.116 per cent; and in this and the other experiments the quantity of sugar excreted was high in comparison with the hyperglycemia. With increasing severity of diabetes, the dogs were kept on protein-fat diets, and accordingly showed uniformly high renal thresholds.[8] Thus, after discontinuance of injections, in Table XXVII there was a negative sugar reaction in the final urine sample with the plasma sugar 0.208 to 0.226 per cent. In Table XXX, Period A was performed while the dog was aglycosuric on bread diet, and there was faint glycosuria at the close with plasma sugar about 0.130 per cent; Period B was performed (November 19) after a change to protein-fat diet had become necessary on October 10, and at the close there was faint glycosuria while the plasma sugar was falling from 0.322 to 0.156 per cent, and none while it was falling from 0.156 to 0.147 per cent; Period C was performed after continuance of protein-fat diet to the following February, and in the final period there was faint glycosuria while the plasma sugar was falling from 0.294 to 0.170 per cent. Dog D4-69 (Table XXXI) was an animal with one kidney, which at different times showed plasma sugar of 0.256 to 0.264 per cent without glycosuria. Also in these animals the quantitative sugar excretion was generally low in comparison with the hyperglycemia. The writer previously[9] suggested fallacies in the apparent exact-

[8] Data will be presented in Paper II of this series. Allen, F. M., *J. Biol. Chem.*, 1920, xliii (in press).

[9] Allen, F. M., Studies concerning glycosuria and diabetes, Cambridge, 1913, 53, 54.

ness of intravenous tolerance tests, particularly the abnormal prominence of the factor of renal permeability in them. Woodyatt and his collaborators have adopted the rate of utilization as a standard, and have striven to avoid the errors of Blumenthal's "saturation limit," but their publications contain no records of tests upon nephritics or others with elevated renal thresholds.

(*b*) The lowered tolerance of the animals which were diabetic or near to diabetes was invariably manifest in their greater hyperglycemia. Three types of plasma sugar curves may be distinguished in the longer injections. First, the normal curve seems to be a plateau which is maintained with only accidental irregularities for as long as 10 hours, as in Table III. A greater number of experiments would have been desirable to establish this as a general rule and exclude exceptions, such as a possible falling tendency of the curve indicating improved utilization due to some reaction of the pancreas or general organism; but Woodyatt's experience that glycosuria rises for the first few hours and then maintains a constant level is opposed to any such reaction of improved utilization. Second, it was noticed[1] that at certain stages of partial pancreatectomy or diabetes there is some kind of reaction which increases the apparent tolerance for glucose given by stomach or subcutaneously, and the microscopic studies also prove that the pancreatic islands can be driven to overfunction by carbohydrate excess. Occasional animals in the proper stage of mild diabetes showed such a reaction in the intravenous tests; *e.g.*, in Table XX a plasma sugar plateau of about 0.2 per cent was maintained over 2 hours, and then fell to 0.128 and 0.112 per cent; and in the longer experiment of Table XXX, Period A, this was more marked, for the plasma sugar which rose as high as 0.715 per cent at the close of the 2nd hour declined gradually to 0.125 per cent at the end of the 8th hour. Sometimes the reaction seems to break under longer strain, so that a transitory fall of plasma sugar concentration is followed by a secondary rise, as in Table XV, Period A. These declines were not explainable by changes of sugar excretion. Third, this power of the pancreas remnant to react to carbohydrate excess is lost in the more severe stages of diabetes. The progress of this loss is seen in Periods B and C of Table XXX; and the other animals with the more severe grades of diabetes show merely an excessive hyperglycemia with no tendency to decline.

(*c*) The combined consideration of the glycosuria and the blood sugar concentration makes the intravenous method sufficiently accurate for practical tests of tolerance, and it holds a position of special usefulness in excluding irregularities of absorption, notably in such conditions as thyroid and pituitary deficiency. Outside of such special conditions, other factors seem to predominate over any possible irregularities of absorption, and it is doubtful if the intravenous method can show the finer gradations of tolerance such as are revealed by the alimentary or subcutaneous administration of glucose. Any lowering of assimilation demonstrable by the intravenous method in this series was fully obvious in feeding tests. On the other hand, Table XVI shows a dubious outcome of the intravenous test in an animal which was very close to diabetes; and in Table XV the test is still more doubtful in an animal possessing only $\frac{1}{3}$ to $\frac{1}{4}$ of the pancreas, in which the results of alimentary or subcutaneous tests are usually plain. It is unfortunate that further tests could not be performed upon animals possessing larger fractions of pancreas. The reasons for the differences between the different modes of administration will receive fuller discussion at the close of this series of papers.

3. Water Balance.—A. Body temperature.—A slight elevation of rectal temperature was the rule in the experiments, as shown in Table IV and incidental observations in Tables XIII, XV, and XVII. The dogs were comfortable and refused offered water, with the single exception in Table XX. Any serious desiccation is therefore excluded.

B. Hydremia.—Hemoglobin estimations by the Fleischl-Miescher method were performed in three normal (Tables II, IV, and XIV) and seven partially depancreatized dogs (Tables XV to XIX, XXVII, and XXVIII). Some of the dogs were anemic from cage life and repeated bleeding experiments, but not to any degree apparently invalidating the experimental results. A fall in hemoglobin during the injection period was the rule, sometimes with a return to the original level at the close and sometimes not. One exception was seen in the rise of hemoglobin in the diabetic dog in Table XXVIII, Period B. There was no uniform relation with the plasma sugar level and no special difference between diabetic and non-diabetic animals. This behavior of the hemoglobin is to be expected when the blood is diluted by

injection of an aqueous solution, and the determinations serve chiefly to prove that the variations in plasma sugar were not explainable by dilution or concentration of the blood. The plasma bicarbonate concentration fell during the injection period in Table XV, Period C, though the fat absorption is a possible factor here; but in Table IV, Period A, it actually rose.

C. Diuresis.—The urine was regularly less than the quantity of solution injected, and there was more or less antidiuretic action of glucose, in the sense of retention of water in the blood or in the body, in both normal and diabetic animals. On the other hand, in one diabetic animal (Table XXVII) a comparison was made between 0.9 per cent saline and 5 per cent glucose in 0.9 per cent saline. Here the hydremia was less and the urine volume greater with the glucose solution, so that in this sense glucose was a diuretic.

In relation to all the above observations, notice should be taken of one fact to avoid confusion in interpretation. Even in severe diabetes, if the tolerance has been conserved so that the greater part of a single dose of sugar is assimilated in a manner approaching the normal, the behavior in other particulars, such as diuresis, should likewise resemble the normal. The similarity of behavior of the diabetic and non-diabetic animals in this series conforms to this expectation.

4. Influence of Protein and Fat Feeding.—In connection with the question whether the endocrine pancreatic function is directly and primarily concerned with carbohydrate metabolism alone or also with protein and fat metabolism, it was desired to perform comparative assimilation tests in fasting and fed animals. Later papers in this *Journal* will corroborate existing evidence that fat feeding causes no immediate glycosuria or hyperglycemia; but in the more severe stages of diabetes protein feeding causes prompt and marked elevations of blood sugar. The greatest importance would therefore be attached to any positive results in normal and mildly diabetic animals, in which protein alone causes no hyperglycemia. In a previous experience[10] feeding tests had shown no important differences of tolerance for starchy foods alone or with protein, but a control with intravenous glucose tests was desired

[10] Allen, F. M., *J. Exp. Med.*, 1920, xxxi, 401.

to exclude possible alterations of absorption. The normal dog in Table II was given identical intravenous glucose injections, Period A fasting and Period B 5 hours after feeding 500 gm. of beef lung. The glycosuria was practically identical in the two instances, and the hyperglycemia was slightly greater in the fasting condition, especially in the early part of the test (probably a slight suggestion of "hunger glycosuria"). The partially depancreatized non-diabetic dog in Table XV was similarly tested fasting, after protein, and after fat feeding. Here also the plasma sugar reached its highest concentration of 0.208 per cent at the end of the 1st hour in the fasting period. Otherwise the plasma sugar levels did not differ appreciably and the glycosuria was practically identical. The mildly diabetic dog in Table XVII was tested first in Period A fasting; then Period B with lard feeding showed distinctly higher hyperglycemia and glycosuria; then Period C with control feeding of clay (swallowed readily in the form of soft molded balls placed in the throat) showed an equal hyperglycemia but less glycosuria; then Period D with lard feeding showed a higher hyperglycemia than before; finally Period E with protein feeding showed the highest hyperglycemia of all. Circumstances prevented a final fasting test, but the record probably indicates merely a gradual aggravation of the diabetes and cannot be regarded as proof of an influence of fat or protein feeding upon the sugar assimilation. It may be mentioned incidentally that ingestion of anything seems sometimes to raise the diabetic blood sugar, as seen in Periods B, C, and E, and the effect is not purely psychic as shown by the negative effect of tantalizing in Period E. The mildly diabetic dog in Table XIX showed no more than accidental variations in hyperglycemia and glycosuria when tested fasting or after protein or fat feeding. The greatest question was raised by the severely diabetic dog in Table XXVIII, which was known from other tests[11] to be subject to marked hyperglycemia from protein feeding alone. Here the variations in glycosuria were trivial, and the hyperglycemia was greatest in the fasting test (Period A), being slightly lower after protein and markedly lower after fat feeding. The vomiting of undigested lung by this dog after the close of the injections, of fat by the dog in Table XVII, Period D, and of clear water in

[11] Allen, F. M., *Am. J. Med. Sc.*, 1917, cliii, 363, 365.

the fasting period of Table XIV, raises the question whether intravenous glucose injections interfere with alimentary absorption. There was marked milkiness of the plasma in Table XV, Period C, but little or none in the other fat-feeding experiments. The experiments at least afford no evidence of a direct participation of the pancreatic hormone in protein or fat metabolism, but their decisiveness is open to question.

TABLE I.

Dog F6-18.

Weight 11 kg. Intravenous glucose injections, 1 gm. per kg. per hr. in 10 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
Dec. 20	3.25 p.m.	27	0	0.086	3.50 p.m. started injections.
	4.45 "	18	0.56	0.109	
	5.45 "	8	0.77	0.118	5.35 p.m. finished injections.
	7.10 "	56	0	0.112	
	8.10 "	40	0	0.109	Total solution injected.... 220 cc.
					Total glucose injected.... 22 gm.
					Total urine excreted ..... 122 cc.
					Total glucose excreted.... 0.16 gm.
					Per cent of dose..... 0.73 per cent.

TABLE II.

Dog C3-93.

Weight 15.5 kg. Normal. Intravenous glucose injections, 1 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine.				Plasma sugar.		Hemoglobin.		Remarks.
		Volume.		Glucose.		A	B	A	B	
		A	B	A	B					
1916		*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
May 1	9.00 a.m.						0.117		105	Fed 500 gm. lung.
Period A.	11.00 "						0.114		98	
May 31	2.00 p.m.			0	0	0.133	0.111	105	100	Started injections.
Period B.	2.20 "	5	19	2.08	0.29	0.153	0.112	92	87	A = Fasting.
	2.40 "	8	18	1.64	0.82	0.132	0.116	85		B = After feeding 500 gm. lung.
	3.00 "	8	20	1.05	0.80	0.095	0.123	89		
	3.20 "	4	13	1.72	0.97	0.119	0.084	98	100	
	3.40 "	5	45	1.52	Faint.	0.103	0.080	104	100	
	4.00 "	6	70	1.39	"	0.098	0.099	106	102	
	4.20 "	13	12	0.80	0.54	0.092	0.128	110	98	
	4.40 "		31		0.75	0.143	0.114	111	100	Finished injections.
	5.00 "	4	15	0.74	0.68	0.161	0.118	105	100	
	5.30 "	18		0		0.105	0.093	104	95	
	7.00 "	58	190	0	Faint.	0.104	0.095	105		

Periods A and B.
- Total solution injected ..... 155 cc.
- " glucose " ..... 31 gm.

Period A.
- Total urine excreted ..... 129 cc.
- " glucose " ..... 0.67 gm.
- Per cent of dose ..... 2.16 per cent.

Period B.
- Total urine excreted ..... 433 cc.
- " glucose " ..... 0.89 gm.
- Per cent of dose ..... 2.87 per cent.

TABLE III.

Dog F6-03.

Weight 20 kg. Intravenous glucose injections, 1 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
Nov. 19	10.45 a.m.	70	0	0.119	11.10 a.m. started injections.
	12.05 p.m.	12	2.06	0.149	
	1.05 "	4	2.12	0.185	
	2.05 "	21	2.38	0.172	Plasma sugar immediately following injection 0.457 per cent.
	3.05 "	15	0.76	0.147	
	4.05 "	124	0.35	0.147	
	5.05 "	65	0.52	0.159	
	6.05 "	77	0.47	0.200	
	7.05 "	92	0.38	0.175	
	8.05 "	86	0.41	0.164	
	9.05 "	78	0.23	0.196	8.55 p.m. finished injections.
	10.00 "	35	Faint.	0.113	
	11.00 "	39	0	0.110	Total solution injected....1,000 cc. Total glucose injected... 200 gm. Total urine excreted..... 648 cc. Total glucose excreted.... 2.95 gm. Per cent of dose..... 1.48 per cent.

TABLE IV.

Dog C3-47.

Weight 15 kg. Normal. Comparative intravenous glucose injections of 1 and 1.5 gm. per kg. per hr. (2 injections per hr.).

Date.	Time.	Urine.				Plasma sugar.		CO_2		Hemoglobin.		Temperature.		Remarks.
		Volume.		Glucose.		A	B	A	B	A	B	A	B	
		A	B	A	B									
1916		*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*per cent*	*°F.*	*°F.*	
Jan. 21	1.30 p.m.	150	171	0	0	0.120	0.133	50		121	100	101.8	101.4	1.40 p.m. started
Period	2.00 "	25	40	(?)	1.56	0.173	0.232	55.7		82	80	102	101.6	injections.
A.	2.30 "	69	145	0	0.32	0.136	0.356	54.8		81	83	102	101.6	
Jan. 27	3.00 "	119	245	0	Very	0.154	0.130*	55.7		79	103*	102.2	102.1	3.06 p.m. finished
Period					faint.									injections.
B.	3.30 "	176	50	0	0.37	0.125	0.196	59.5		84	88	102	102	
	3.40 "	110	95	0	0	0.114	0.128	59.5		84	87	101.6	101.8	

A. 1.0 gm. per kg. per hr. in 5 per cent solution.
Total solution injected..... 600 cc.
" glucose " ..... 30 gm.
" urine excreted..... 499 cc.
" glucose " ..... 0 gm.
Per cent of dose..... 0 per cent.

B. 1.5 gm. per kg. per hr. in 7.5 per cent solution.
Total solution injected..... 600 cc.
" glucose " ..... 45 gm.
" urine excreted..... 575 cc.
" glucose " ..... 1.02 gm.
Per cent of dose..... 2.27 per cent.

* This simultaneous break in the sugar and hemoglobin curves is unexplained.

TABLE V.

Dog F6-33.

Weight 12 kg. Intravenous glucose injections, 1.5 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
May 22	4.40 p.m.	20	0	0.093	Started injections.
Period A.	5.40 "	27	1.93	0.088	
	6.40 "	22	1.37	0.085	6.20 p.m. finished injections.
	7.40 "	32	Faint.	0.115	
	8.40 "	15	"	0.114	Total solution injected..... 180 cc.
					Total glucose injected..... 36 gm.
					Total urine excreted..... 86 cc.
					Total glucose excreted..... 0.82 gm.
					Per cent of dose..... 2.28 per cent.
June 13	4.30 p.m.	41	0	0.111	Started injections.
Period B.	5.30 "	14	2.94	0.170	
	6.30 "	12	2.93	0.132	
	7.30 "	14	Faint.	0.128	7.10 finished injections.
	8.30 "	56	0	0.110	
					Total solution injected..... 270 cc.
					Total glucose injected..... 54 gm.
					Total urine excreted..... 96 cc.
					Total glucose excreted..... 0.76 gm.
					Per cent of dose..... 1.41 per cent.

TABLE VI.

Dog F6-84.

Weight 7.6 kg. Intravenous glucose injections, 1.5 gm. per kg. per hr. (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
June 12	4.30 p.m.	25	0	0.149	Started injections.
	5.30 "	14	3.23	0.208	
	6.30 "	17	3.22	0.176	6.10 p.m. finished injections.
	7.30 "	32	Faint.	0.102	
	8.30 "	20	0	0.103	Total solution injected..... 114 cc.
					Total glucose injected..... 22.8 gm.
					Total urine excreted..... 83 cc.
					Total glucose excreted.... 1.0 gm.
					Per cent of dose..... 4.40 per cent.

TABLE VII.

Dog F6-83.

Weight 9 kg. Intravenous glucose injections, 1.5 gm. per kg. per hr. (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
May 23	4.20 p.m.	25	0	0.109	5.40 p.m. started injections.
	6.40 "	52	3.70	0.159	
	7.40 "	58	2.86	0.152	
	8.40 "	124	2.33	0.218	8.20 p.m. finished injections.
	9.40 "	38	Faint.	0.102	
	10.40 "	12	Very faint.	0.114	Total solution injected..... 203 cc.
					Total glucose injected..... 40.5 gm.
					Total urine excreted..... 284 cc.
					Total glucose excreted..... 6.47 gm.
					Per cent of dose..... 1.60 per cent.

TABLE VIII.

Dog F6-84.

Weight 9 kg. Intravenous glucose injections, 1 gm. per kg. per hr. (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
May 23	4.35 p.m.	18	0	0.086	5.15 p.m. started injections.
	6.15 "	50	2.63	0.175	
	7.15 "	26	2.80	0.179	
	8.15 "	90	1.50	0.104	7.55 p.m. finished injections.
	9.15 "	63	Faint.	0.075	
	10.15 "	25	Very faint.	0.089	Total solution injected..... 203 cc Total glucose injected..... 40.5 gm. Total urine excreted..... 244 cc. Total glucose excreted..... 3.41 gm. Per cent of dose..... 8.40 per cent.

TABLE IX.

Dog G7-39.

Weight 11 kg. Intravenous glucose injections, 1.5 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks
		Volume.	Glucose.		
1918		*cc.*	*per cent*	*per cent*	
July 10	1.50 p.m.	15	0	0.118	Started injections.
	2.50 "	10	2.00	0.167	
	3.50 "	5	2.80	0.137	
	4.50 "	148	0.26	0.232	4.35 p.m. finished injections.
	5.50 "	44	Very faint.	0.085	
	6.50 "	66	0	0.131	Total solution injected..... 248 cc. Total glucose injected..... 49.5 gm. Total urine excreted..... 273 cc. Total glucose excreted..... 0.73 gm. Per cent of dose..... 1.50 per cent.

TABLE X.

Dog F6-31.

Weight 18 kg. Intravenous glucose injections, 1.5 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1918		*cc.*	*per cent*	*per cent*	
April 5	2.30 p.m.	58	0	0.135	Started injections.
	3.30 "	62	0.66	0.182	
	4.30 "	106	0.22	0.132	
	5.30 "	80	0.28	0.192	5.10 p.m. finished injections.
	6.30 "	50	0.59	0.152	
	7.30 "	50	0.12	0.106	Total solution injected..... 405 cc. Total glucose injected.... 81 gm. Total urine excreted..... 348 cc. Total glucose excreted.... 1.22 gm. Per cent of dose ..... 1.50 per cent.

TABLE XI.

Dog G7-69.

Weight 8 kg. Intravenous glucose injections, 1.5 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1918		*cc.*	*per cent*	*per cent*	
July 10	1.55 p.m.	14	0	0.122	Started injections.
	2.55 "	23	0.65	0.122	
	3.55 "	33	0.48	0.164	
	4.55 "	60	0.23	0.156	4.35 p.m. finished injections.
	5.55 "	56	Very faint.	0.147	
	6.55 "	20	0	0.102	Total solution injected.... 180 cc. Total glucose injected.... 36 gm. Total urine excreted..... 192 cc. Total glucose excreted.... 0.45 gm. Per cent of dose..... 1.28 per cent.

TABLE XII.

Dog F6-32.

Weight 14 kg. Intravenous glucose injections, 1.5 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1918		*cc.*	*per cent*	*per cent*	
April 5	2.20 p.m.	72	0	0.120	Started injections.
	3.20 "	46	0.48	0.204	
	4.20 "	47	0.28	0.192	
	5.20 "	132	0.40	0.208	
	6.20 "	70	0.28	0.250	6.05 p.m. finished injections.
	7.20 "	38	0.23	0.109	
					Total solution injected..... 420 cc.
					Total glucose injected..... 84 gm.
					Total urine excreted..... 333 cc.
					Total glucose excreted.... 1.17 gm.
					Per cent of dose..... 1.40 per cent.

TABLE XIII.

Dog F6-05.

Weight 15 kg. Intravenous glucose injections, 1.5 gm. per kg. per hr. in 10 per cent solution (4 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
Dec. 4	10.00 a.m.	50	0	0.109	Started injections.
	11.00 "	148	Faint.	0.118	
	12.00 "	256	Slight.	0.123	
	1.00 p.m.	360	0.18	0.143	
	2.00 "	180	0.78	0.170	
	3.00 "	132	0.32	0.151	3.20 p.m. temperature 39.6°C.
	4.00 "	330	0.14	0.104	
	5.00 "	146	0.19	0.104	6.30 p.m. temperature 39.1°C.
	6.00 "	225	0.25	0.105	6.40 p.m. finished injections.
	7.00 "	146	0.15	0.119	
	8.00 "	40	Very faint.	0.109	Total solution injected..... 2,027 cc. Total glucose injected... 202.7 gm. Total urine excreted... 1,963 cc. Total glucose excreted..... 3.68 gm. Per cent of dose..... 1.88 per cent.

TABLE XIV.

Dog C3-92.

Weight 18 kg. Normal. Continuous intravenous glucose injection, 2 gm. per kg. per hr. in 5 per cent solution.

Date.	Time.	Urine.		Plasma sugar.	Hemoglobin.	Remarks.
		Volume.	Glucose.			
1916		*cc.*	*per cent*	*per cent*	*per cent*	
Aug. 4	2.25 p.m.	20	0	0.114	90	2.30 p.m. started injection.
	3.30 "	85	3.00		77	Vomited during experiment 300 cc. of clear fluid, sugar-free.
	4.30 "	90	2.50	0.330	78	
	5.30 "	320	0.50	0.300	77	
	6.30 "	200	Faint.			Finished injection.
	7.30 "	135	0	0.111		
						Total solution injected... 2,160 cc.
						Total glucose injected... 108 gm.
						Total urine excreted... 850 cc.
						Total glucose excreted... 6.4 gm.
						Per cent of dose..... 5.90 per cent.

TABLE XV.

Dog C8-88.

Weight 10 kg. Non-diabetic; ¼ to ⅓ of pancreas present.* Intravenous glucose injections, 1 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine. Volume.	Urine. Glucose.	Plasma sugar.	Hemoglobin.	CO_2	Remarks.
1918		*cc.*	*per cent*	*per cent*	*per cent*	*vol. per cent*	
May 8	2.00 p.m.		0	0.117	90		2.02 p.m. injections started.
Period	2.20 "	4	1.14	0.170	83		
A.	2.40 "	3	1.82	0.160	90		
	3.00 "	4	2.32	0.208	88		
	3.20 "			0.123	84		
	3.40 "	8	1.85	0.105	86		Low renal threshold.
	4.00 "			0.147			Temperature 102.9°F.
	4.20 "	8	1.47	0.143	82		Note secondary rise of plasma sugar.
	4.40 "	9	1.93	0.164	86		4.42 p.m. finished injections.
	5.00 "	19	0.22	0.122			Temperature 102°F.
	5.30 "	22	Faint.	0.116	90		
	6.30 "	24	Doubtful.				Total solution injected. 150 cc. Total glucose injected... 30 gm. Total urine excreted.. 118 cc. Total glucose excreted.. 0.66 gm. Per cent of dose.... 2.20 per cent.
	7.15 "	17	0	0.102	87		
June 2	2.05 p.m.		0	0.133	95		8.00 a.m. fed 500 gm. of lung.
Period	2.20 "	16	0.51	0.164	74		2.05 p.m. started injections.
B.	2.40 "			0.164	76		
	3.00 "	18	0.94	0.149	74		
	3.20 "	8	1.10	0.145	85		
	3.40 "	5	1.38	0.167	87		
	4.00 "	15	1.06	0.132	85		
	4.20 "	4	1.54	0.167	90		

*All operations were performed under ether anesthesia.

TABLE XV—*Concluded.*

Date.	Time.	Urine.		Plasma sugar.	Hemoglobin.	CO_2	Remarks.
		Volume.	Glucose.				
1916		*cc.*	*per cent*	*per cent*	*per cent*	*vol. per cent*	
June 2 Period B.— Continued.	4.40 p.m.	5	0.88	0.164			4.42 p.m. finished injections.
	5.00 "	15	0.23	0.102			
	5.30 "	92	0	0.087			Total solution injected. 150 cc.
	6.30 "	21	0				Total glucose injected... 30 gm.
	7.15 "	43	0	0.118			Total urine excreted... 238 cc.†
							Total glucose excreted.. 0.60 gm.
							Per cent of dose.... 2.0 per cent.
May 25 Period C.	3.00 p.m.		0	0.102	90	55.8	9.30 a.m. fed 200 gm. of lard.
	3.20 "	4	Faint.	0.161	88	54.8	3.00 p.m. started injections.
	3.40 "	6	0.76	0.147	85	50.4	Heavy lipemia.
	4.00 "	3	0.54	0.156	70	54.8	Moderate "
	4.20 "	4	1.24	0.161	65	50.4	
	4.40 "			0.189	74	56.2	Finished injections.
	5.00 "	3	1.37	0.185	74	56.2	
	5.20 "			0.128	65	51.4	Heavy lipemia.
	5.40 "	15	0.26	0.143	62	56.2	" "
	6.00 "	2	1.33	0.143	70	56.2	
	6.30 "	15	Faint.	0.083	75	56.2	Total solution injected.. 90 cc.
	7.30 "	5	Very faint.				Total glucose injected.... 20 gm.
	8.15 "	13	0	0.100	71	55.8	Total urine excreted. 70 cc.
							Total glucose excreted... 0.34 gm.
							Per cent of dose..... 1.70 per cent.

† The surplus water excreted is presumably derived from the food.

TABLE XVI.

Dog B2-00.

Weight 14 kg. Partially depancreatized non-diabetic. Two subsequent operations, removing first 0.8 gm. and then 0.1 gm. of pancreas, were required to produce diabetes. Intravenous glucose injections, 1.5 gm. per kg. per hr. in 5 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Hemoglobin.	Remarks.
		Volume.	Glucose.			
1916		*cc.*	*per cent*	*per cent*	*per cent*	
Jan. 31	1.30 p.m.		0	0.105	112	1.45 p.m. started injections.
	2.00 "	60	0.36			
	2.20 "	90	0.95	0.288	98	
	2.40 "	100	0.50	0.145	91	3.00 p.m. finished injections.
	3.30 "	175	0	0.091	93	
	3.45 "	25	0	0.088	85	Total solution injected..... 560 cc.
	4.00 "	30	0	0.098	80	Total glucose injected..... 28 gm.
						Total urine excreted..... 480 cc.
						Total glucose excreted..... 1.57 gm.
						Per cent of dose. 5.60 per cent.

TABLE XVII.

Dog B2-01.

Weight 14 kg. Partially depancreatized. Mildly diabetic. Continuous intravenous glucose injections, 1.2 gm. per kg. per hr. in 5 per cent solution.

Date.	Time.	Urine.		Plasma sugar.	Hemoglobin.	Remarks.
		Volume.	Glucose.			
1916		*cc.*	*per cent*	*per cent*	*per cent*	
May 17	2.25 p.m.	25	0	0.116	107	2.30 p.m. started injections.
Period	3.00 "	76	2.44	0.228	103	
A.	4.30 "	190	1.47	0.286	95	Temperature 39.2°C.
	5.00 "	160	1.21	0.238	83	
	5.30 "	95	1.06	0.222	90	Finished injections.
	6.30 "	31	0.34	0.084	99	Note low renal threshold.
	7.10 "	19	Slight faint.	0.087	85	
	8.30 "	69		0.116	90	Total solution injected. 1,008 cc.
						Total glucose injected.. 50.4 gm.
						Total urine excreted.. 640 cc.
						Total glucose excreted.. 7.64 gm.
						Per cent of dose. 14.1 per cent.
May 24	8.25 a.m.	50	0	0.087	108	Fed 200 gm. of lard.
Period	2.25 p.m.	2	0	0.122	110	3.00 p.m. started injections.
B.	4.00 "	110	1.89	0.385	102	
	5.00 "	216	2.13	0.435	92	
	6.00 "	350	2.04	0.358	97	Finished injections.
	7.00 "	63	1.73	0.182	98	
	8.00 "	10	0.45	0.147	98	Total solution injected. 1,008 cc.
	9.30 "	7	Faint.	0.147	98	
						Total glucose injected.. 50.4 gm.
						Total urine excreted.. 758 cc.
						Total glucose excreted.. 14.97 gm.
						Per cent of dose. 27.7 per cent.

TABLE XVII—*Continued.*

Date.	Time.	Urine.		Plasma sugar.	Hemoglobin.	Remarks.
		Volume.	Glucose.			
1916		*cc.*	*per cent*	*per cent*	*per cent*	
May 29	8.45 a.m.	20	0	0.102	95	Fed 300 gm. of white clay.
Period	9.45 "			0.120	96	
C.	2.00 p.m.	2	0	0.103	97	2.30 p.m. started injections.
	3.30 "	74	2.04	0.345	74	
	4.30 "	320	2.38	0.500	66	
	5.30 "	278	3.70	0.385	68	Finished injection.
	6.30 "	30	2.22	0.182		Drank 250 cc. of water.
	7.45 "			0.179		" 200 " " "
	9.00 "	16	0.28	0.135	84	
						Total solution injected. 1,008 cc.
						Total glucose injected.. 50.4 gm.
						Total urine excreted.. 710 cc.
						Total glucose excreted.. 9.84 gm.
						Per cent of dose. 18.2 per cent.
June 1	2.40 p.m.			0.104	92	8.00 a.m. fed 200 gm. of lard.
Period	4.00 "	30	3.65	0.357	88	3.00 p.m. started injections. Slight lipemia.
D.	5.00 "	348	2.38	0.371	75	Negative "
	6.00 "	182	2.64	0.417	77	Finished injections. Negative lipemia. Vomited about 25 gm. of lard. Drank 200 cc. of water.
	7.00 "	40	4.17	0.193	88	
	8.00 "	22	Faint.	0.145		
	9.30 "	65	0	0.119	77	Total solution injected. 1,008 cc.
						Total glucose injected.. 50.4 gm.
						Total urine excreted.. 687 cc.
						Total glucose excreted.. 15.86 gm.
						Per cent of dose. 31.5 per cent.

TABLE XVII—*Concluded.*

Date.	Time.	Urine.		Plasma sugar.	Hemoglobin.	Remarks.
		Volume.	Glucose.			
1916		*cc.*	*per cent*	*per cent*	*per cent*	
June 5 Period E.	9.35 a.m.		0	0.093	85	Tantalized with meat for 5 min., not allowed to eat it.
	9.40 "			0.093	87	Fed 500 gm. of lung.
	9.50 "			0.109	82	
	10.10 "			0.119	80	
	10.25 "		0	0.156	78	3.20 p.m. started injections.
	4.20 p.m.	123	3.58	0.358	70	
	5.20 "	232	3.85	0.477	73	
	6.20 "	240	2.18	0.264	72	Finished injections.
	7.20 "	14	0.32	0.122	74	
	8.20 "	19	Faint.	0.182	71	Total solution injected. 1,008 cc.
	9.50 "	19	"	0.121		Total glucose injected.. 50.4 gm.
						Total urine excreted.. 647 cc.
						Total glucose excreted.. 18.61 gm.
						Per cent of dose. 37.0 per cent.

TABLE XVIII.

Dog B2-02.

Weight 10.5 kg. Partially depancreatized. Intravenous glucose injections, 1.5 gm. per kg. per hr. in 5 per cent solution in 0.9 per cent saline (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Hemoglobin.	Remarks.
		Volume.	Glucose.			
1916		*cc.*	*per cent*	*per cent*	*per cent*	
Feb. 2	1.55 p.m.		0	0.111	114	2.15 p.m. started injections.
	2.30 "	10	2.32	0.209	108	
	2.50 "	25	2.38	0.271	106	
	3.10 "	35	2.13	0.250	97	2.55 p.m. finished injections.
	3.25 "	12	0.64	0.200	104	
	3.40 "	10	Faint.	0.137	103	Total solution injected.... 316 cc.
	3.55 "	10	0	0.125	99	Total glucose injected.... 15.8 gm.
						Total urine excreted. 102 cc.
						Total glucose excreted... 1.66 gm.
						Per cent of dose..... 10.5 per cent.

TABLE XIX.

Dog C3-74.

Weight 18 kg. Partially depancreatized. Mildly diabetic. Intravenous glucose injections, 1 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine. Volume.	Urine. Glucose.	Plasma sugar.	Hemoglobin.	Remarks.
1916		*cc.*	*per cent*	*per cent*	*per cent*	
May 8	3.00 p.m.			0.119	88	Started injections.
Period	3.20 "	8	1.82	0.173	91	3.05 p.m. blood taken 2
A.	3.40 "	6	3.60	0.263	91	minutes after finishing in-
	4.00 "	19	1.22	0.286	90	jection; plasma sugar
	4.20 "	10	0.86	0.303	94	0.294 per cent.
	4.40 "	23	0.82	0.312	92	Finished injections.
	5.00 "	22	0.22	0.238	94	
	5.40 "	20	0.31			Total solu-
	6.20 "			0.137	96	tion in-
	6.40 "	20	Faint.			jected... 180 cc.
	7.00 "			0.126	92	Total glu-
	7.40 "	20	Very faint.			cose in- jected... 36 gm.
	8.20 "	20	Very faint.	0.125	93	Total urine excreted. 174 cc.
						Total glu-
						cose ex-
						creted... 0.99 gm.
						Per cent of
						dose..... 3.02 per cent.
May 23	2.10 "		0	0.139	88	10.25 a.m. fed 1,800 gm. of
Period						lung.
B.	2.45 "	20	0.83	0.227	87	
	3.05 "	19	0.95	0.250	87	2.25 p.m. started injections.
	3.25 "	15	1.54	0.264	84	
	3.45 "	18	2.08	0.286	85	
	4.05 "	18	2.38	0.286		Finished injections.
	4.20 "	21	1.93	0.313	86	
	5.00 "	19	0			Total solu-
	5.40 "			0.185	88	tion in-
	6.00 "	49	0			jected... 180 cc.
	6.20 "			0.139	85	Total glu-
	7.10 "	53	0			cose in-
	7.40 "	56	0	0.137	82	jected... 36 gm.
						Total urine excreted. 288 cc.
						Total glu-
						cose ex-
						creted... 1.79 gm.
						Per cent of
						dose..... 4.98 per cent.

TABLE XIX—*Concluded.*

Date.	Time.	Urine.		Plasma sugar.	Hemoglobin.	Remarks.
		Volume.	Glucose.			
1916		*cc.*	*per cent*	*per cent*	*per cent*	
May 12 Period C.	2.00 p.m.		0	0.097	90	10.00 a.m. fed 200 gm. of lard.
	2.20 "	13	0.65	0.151	95	2.00 p. m. started injections.
	2.40 "	15	0.70	0.232	85	
	3.00 "	15	0.66	0.162	88	
	3.20 "	18	0.49	0.167	85	
	3.40 "	14	0.61	0.137	88	Finished injections.
	4.00 "	12	0.65	0.107	85	
	4.20 "	10	0			Total solution injected... 180 cc.
	4.40 "	11	0			
	5.20 "	10	0	0.099	83	
	6.20 "	60	0	0.111	78	Total glucose injected... 36 gm.
	7.20 "	190	0	0.115	89	Total urine excreted. 368 cc.
						Total glucose excreted... 0.54 gm.
						Per cent of dose..... 1.50 per cent.

TABLE XX.

Dog B2-88.

Weight 13 kg. Mild diabetes. Glycosuria absent on protein-fat diets with addition of 100 gm. of bread; present with addition of bread to 200 gm. Intravenous glucose injections, 1 gm. per kg. per hr. in 5 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1913		*cc.*	*per cent*	*per cent*	
Dec. 2	1.20 p.m.		0	0.118	11.40 a.m. drank 500 cc. of water, also at 1.20 p.m.
	1.59 "	120	0	0.200	1.44 p.m. started injections.
	2.20 "	64	0	0.200	
	2.43 "	73	0	0.232	
	3.05 "	100	0	0.192	
	3.38 "	106	0	0.200	
	3.54 "	64	0	0.200	
	4.15 "	90	0	0.200	
	4.37 "	90	0	0.128	
	4.58 "	110	0	0.112	5.08 p.m. finished injections.
	5.24 "	117	0	0.121	
					Total solution injected..... 860 cc.
					Total glucose injected..... 43.3 gm.
					Total urine excreted..... 934 cc.
					Total glucose excreted..... 0 gm.
					Per cent of dose... 0 per cent.

TABLE XXI.

Dog E5-97.

Weight 14 kg. Mild diabetes. Glycosuria absent on diet of beef lung with 100 gm. of bread; present with addition of bread to 200 gm. Intravenous glucose injections, 1 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
Nov. 1	4.05 p.m.		0	0.095	4.20 p.m. started injections.
	5.20 "	No urine.		0.286	
	6.25 "	70	2.44	0.294	6.10 p.m. finished injections.
	7.30 "	No urine.		0.164	
	8.30 "	40	1.47	0.145	Total solution injected..... 140 cc. Total glucose injected..... 28 gm. Total urine excreted..... 110 cc. Total glucose excreted..... 2.30 gm. Per cent of dose. 8.2 per cent.

TABLE XXII.

Dog F6-00.

Weight 20 kg. Mild diabetes. Glycosuria absent on diet of 1 kg. of lung and 100 gm. of bread; heavy on increasing bread to 200 gm. Intravenous glucose injections, 1 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1918		*cc.*	*per cent*	*per cent*	
Feb. 7	2.15 p.m.		0	0.127	2.25 p.m. started injections.
	3.20 "	25	3.64	0.555	
	4.20 "	87	4.98	0.555	
	5.20 "	180	5.89	0.667	5.10 p.m. finished injections.
					Total solution injected..... 300 cc. Total glucose injected..... 60 gm. Total urine excreted..... 292 cc. Total glucose excreted..... 15.84 gm. Per cent of dose.. 26.4 per cent.

TABLE XXIII.

Dog E5-95.

Weight 16 kg. Moderate diabetes. Glycosuria absent on diet of 80 gm. of lung and 100 gm. of suet; present with addition of 50 gm. of bread. Intravenous glucose injections, 1 gm. per kg. per hr. in 20 per cent solution (4 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
Nov. 1	1.20 p.m.		0	0.109	1.40 p.m. started injections.
	2.35 "	22	3.62	0.357	
	3.40 "	90	4.31	0.416	3.25 p.m. finished injections.
	4.40 "	32	5.21	0.200	
	5.40 "	10	1.87	0.218	Total solution injected..... 160 cc.
					Total glucose injected..... 32 gm.
					Total urine excreted..... 154 cc.
					Total glucose excreted..... 6.55 gm.
					Per cent of dose. 20.4 per cent.

TABLE XXIV.

Dog D4-62.

Weight 19 kg. Moderate diabetes. Glycosuria absent on diet of 1 kg. of lung; heavy with the addition of 50 gm. of bread. Intravenous glucose injections, 1 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1916		*cc.*	*per cent*	*per cent*	
Jan. 31	1.45 p.m.		0	0.100	1.50 p.m. started injections.
	2.05 "	10	2.94	0.196	
	2.25 "	21	3.23	0.385	
	2.45 "	25	4.35	0.385	
	3.05 "	21	5.56	0.435	
	3.25 "	28	6.25	0.455	
	3.45 "	27	5.41	0.455	3.50 p.m. finished injections.
	4.05 "	33	4.54	0.416	
	5.05 "	21	3.57	0.384	Total solution injected..... 223 cc.
	6.05 "	4	2.08	0.286	
	7.30 "	7	Faint.	0.137	Total glucose injected..... 44.3 gm.
	10.00 "	10	0	0.088	
					Total urine excreted..... 207 cc.
					Total glucose excreted..... 8.77 gm.
					Per cent of dose..... 19.8 per cent.

TABLE XXV.

Dog D4-84.

Weight 14 kg. Moderate diabetes. Slight glycosuria on 1 kg. of beef lung. Intravenous glucose injections, 1 gm. per kg. per hr. in 10 per cent solution (4 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
Oct. 31	1.30 p.m.			0.154	Started injections.
	2.30 "			0.400	
	3.30 "			0.525	Finished injections.
	4.30 "			0.370	
	5.40 "			0.200	Total solution injected..... 280 cc.
					Total glucose injected..... 28 gm.
					Total urine, in 24 hrs., 390 cc. with 1.6 per cent glucose.
					Total glucose excreted..... 6.24 gm.
					Per cent of dose. 22.2 per cent.

TABLE XXVI.

Dog E5-19.

Weight 9.0 kg. Severe diabetes. Glycosuria absent on diet of 100 gm. of lung and 100 gm. of suet; faint on 200 gm. of lung and 100 gm. of suet; heavy on 300 gm. of lung and 100 gm. of suet. Intravenous glucose injections, 1 gm. per kg. per hr. in 5 per cent solution in 0.9 per cent saline. (4 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
Oct. 31	10.15 a.m.		0	0.133	Started injections.
	11.15 "	Not catheterized.		0.322	
	12.15 p.m.	57	5.10	0.475	Finished injections.
	1.15 "	15	3.29	0.278	
	2.15 "	13	2.16	0.286	Total solution injected..... 420 cc. Total glucose injected... 18 gm. Total urine excreted... 85 cc. Total glucose excreted... 3.7 gm. Per cent of dose..... 20.5 per cent. Glucose in 85 cc. of additional urine up to next morning too little to titrate.

TABLE XXVII.

Dog B2-79.

Weight 14 kg. Partially depancreatized. Severe diabetes. Tolerance 500 gm. of lung. Period A, intravenous glucose injections, 1.5 gm. per kg. per hr. in 5 per cent solution in 0.9 per cent saline (3 injections per hr.). Period B, intravenous saline injections, total received 420 cc. (3 injections per hr.).

Date.	Time.	Urine.				Plasma sugar.		Hemoglobin.		Temperature.		Remarks.
		Volume.		Glucose.		A	B	A	B	A	B	
		A	B	A	B							
1915		*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*°F.*	*°F.*	
Dec. 15	1.30 p.m.	31	50	0	0	0.135	0.167	104	113	101.4	101.6	1.50 p.m. started injec-
Period A.	2.10 "	15	9	1.88	0	0.294	0.147		104	101.1	101.3	tions.
Dec. 27	2.30 "	65	15	2.14	0	0.400	0.139	102	98	100.9	101.7	Finished injections.
Period B.	2.50 "	108	38	2.11	0	0.400	0.139	93	81	100.8	101.6	
	3.10 "	27	13	2.31	0	0.358	0.139	94	81	100.8	101.2	
	3.30 "	13	10	1.78	0	0.250	0.162	85	90	101.1	101.3	
	3.45 "	17	14	0.46	0	0.264	0.167	93	80	101.4	101.4	
	4.00 "	26	20	Very faint.	0	0.208	0.152	90	82	101.4	101.2	
	4.15 "	40	21	0	0	0.226	0.183	96		102.2	101.7	

A. Total solution injected..... 420 cc.
" glucose " ..... 21 gm.
" urine excreted..... 311 cc.
" glucose " ..... 4.9 gm.
Per cent of dose..... 23.3 per cent.

B. Total solution injected..... 420 cc.
" urine excreted..... 140 "

TABLE XXVIII.

Dog C3-86.

Weight 13 kg. Partially depancreatized. Severely diabetic. Intravenous glucose injections, 1 gm. per kg. per hr. in 20 per cent solution (3 injections per hr.).

Date.	Time.	Urine. Volume.	Urine. Glucose.	Plasma sugar.	Hemoglobin.	Remarks.
1916		*cc.*	*per cent*	*per cent*	*per cent*	
May 9	2.55 p.m.		0	0.128	99	Started injections.
Period	3.15 "	8	0.57	0.130	95	
A.	3.35 "	3	1.54	0.200	97	
	3.55 "	10	1.08	0.270	90	
	4.15 "	3	3.45	0.227	84	
	4.35 "	3	2.04	0.232	76	Finished injections.
	4.55 "	4	1.76	0.185	74	
	5.30 "	55	0			Total solution injected..... 130 cc.
	5.45 "			0.123	90	
	7.00 "	91	0	0.099	99	Total glucose injected... 26 gm.
						Total urine excreted... 177 cc.
						Total glucose excreted... 0.44 gm.
						Per cent of dose..... 1.69 per cent.
May 15						10.30 a.m. fed 200 gm. of lard.
Period	3.00 "		0	0.098	90	Started injections.
B.	3.20 "	6	1.79	0.170	90	
	3.40 "	11	0.95	0.170	80	
	4.00 "	11	1.20	0.164	94	
	4.20 "	4	2.15	0.145	100	
	4.40 "	3	2.09	0.137	100	Finished injections.
	5.00 "	5	1.61	0.125	100	
	5.50 "	8	Slight.	0.116	96	Total solution injected..... 130 cc.
	7.00 "	15	0	0.100	89	
	7.30 "	12	0			Total glucose injected... 26 gm.
						Total urine excreted... 75 cc.
						Total glucose excreted... 0.57 gm.
						Per cent of dose..... 2.19 per cent.

TABLE XXVIII—*Concluded.*

Date.	Time.	Urine.		Plasma sugar.	Hemoglobin.	Remarks.
		Volume.	Glucose.			
1916		*cc.*	*per cent*	*per cent*	*per cent*	
May 22	11.25 a.m.			0.119	88	Fed 1,200 gm. of lung.
Period	1.50 p.m.	72	0	0.123	88	Started injections.
C.	2.15 "	11	2.38	0.164	88	
	2.35 "	16	2.27	0.185	77	
	2.55 "	9	4.16	0.233	81	
	3.15 "	15	3.85	0.204	80	
	3.35 "	12	3.03	0.200	70	Finished injections.
	3.55 "	12	1.52	0.161	89	
	4.40 "	15	Faint.	0.125	93	4.40 p.m. vomited 400 gm. of undigested lung.
	6.25 "	75	0	0.095	100	
						Total solution injected... 130 cc.
						Total glucose injected. 26 gm.
						Total urine excreted 165 cc.
						Total glucose excreted 2.11 gm.
						Per cent of dose..... 8.10 per cent.

TABLE XXIX.

Dog E5-90.

Weight 10 kg. Period A, very mild diabetes. Glycosuria absent on diet of bread and soup; continuously heavy with addition of 100 gm. of glucose. Period B, moderate diabetes. Glycosuria absent on diet of lung and suet; heavy with addition of 200 gm. of bread. Intravenous glucose injections, 1 gm. per kg. per hr. in 10 per cent solution (4 injections per hr.).

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
Oct. 25	1.45 p.m.		0	0.164	1.55 p.m. started injections.
Period	2.50 "	5	0.68	0.333	
A.	3.50 "	14	0.57	0.323	4.40 p.m. finished injections.
	4.50 "	95	Faint.	0.213	
	6.15 "			0.119	
					Total solution injected.... 300 cc.
					Total glucose injected.... 30 gm.
					Total urine excreted.... 114 cc.
					Total glucose excreted.... 0.11 gm.
					Per cent of dose..... 0.37 per cent.
Dec. 18	2.45 "		0	0.122	3.00 p.m. started injections.
Period	4.00 "	14	2.25	0.384	
B.	5.00 "	50	4.00	0.500	5.45 p.m. finished injections.
	6.00 "	26	2.60	0.455	
					Total solution injected.... 300 cc.
					Total glucose injected.... 30 gm.
					Total urine excreted.... 90 cc.
					Total glucose excreted.... 3.0 gm.
					Per cent of dose..... 10 per cent.

TABLE XXX.

Dog D4-52.

Weight, Period A, 10.75 kg.; Period B, 11.7 kg.; Period C, 12 kg. Glycosuria absent on bread and soup diet to Oct. 10, 1917. Absent thereafter on 500 gm. of lung and 100 gm. of suet, with hyperglycemia. Intravenous glucose injections, 1 gm. per kg. per hr. in 10 per cent solution (4 injections per hr.), based on normal weight of 12 kg.

Date.	Time.	Urine.		Plasma sugar.	Remarks.
		Volume.	Glucose.		
1917		*cc.*	*per cent*	*per cent*	
Aug. 6 Period A.	Before injection.		0	0.218	
	End 1st hr.	16	2.64	0.500	
	" 2nd "	16	5.08	0.715	
	" 3rd "	54	2.84	0.475	Plasma sugar
	" 4th "	52	1.54	0.590	immediately
	" 5th "	76	0.74	0.415	after injection. 0.770 per cent.
	" 6th "	78	1.81	0.270	Total solution
	" 7th "	94	1.45	0.202	injected..... 828 cc.
	" 8th "	82	0.82	0.125	Total glucose
	1 hr. after injection.	53	0.19	0.128	injected..... 96 gm. Total urine excreted..... 563 cc.
	2 hrs. after injection.	12	Faint.	0.130	Total glucose excreted..... 7.8 gm.
	3 hrs. after injection.	29	0	0.133	Per cent of dose. 8.1 per cent.
Nov. 19 Period B.	Before injection.		0	0.169	
	End 1st hr.	36	1.93	0.555	
	" 2nd "	68	3.08	0.555	
	" 3rd "	82	1.49	0.435	
	" 4th "	132	0.79	0.370	
	" 5th "	98	0.39	0.370	Total solution
	" 6th "	126	0.28	0.356	injected..... 1,200 cc.
	" 7th "	127	0.55	0.370	Total glucose
	" 8th "	105	0.39	0.370	injected..... 120 gm.
	" 9th "	105	0.36	0.384	Total urine ex-
	" 10th "	101	0.42	0.322	creted..... 1,035 cc.
	1 hr. after injection.	45	Faint.	0.156	Total glucose excreted.... 7.7 gm.
	2 hrs. after injection.	10	0	0.147	Per cent of dose..... 6.4 per cent.

TABLE XXX—*Concluded.*

Date.	Time.	Urine. Volume.	Urine. Glucose.	Plasma sugar.	Remarks.
1918		*cc.*	*per cent*	*per cent*	
Feb. 19 Period C.	Before injection.		0	0.145	
	End 1st hr.	20	4.77	0.417	
	" 2nd "	55	5.13	0.476	
	" 3rd "	114	3.39	0.500	
	" 4th "	109	3.45	0.525	Total solution injected..... 960 cc.
	" 5th "	102	3.40	0.500	
	" 6th "	140	2.78	0.475	Total glucose injected..... 96 gm.
	" 7th "	90	4.35	0.455	
	" 8th "	87	3.23	0.384	Total urine excreted..... 778 cc.
	1 hr. after injection.	43	0.74	0.294	Total glucose excreted..... 25.7 gm.
	2 hrs. after injection.	19	Very faint.	0.170	Per cent of dose..... 26.8 per cent.

TABLE XXXI.

Dog D4-69.

Glycosuria absent on bread and soup to Oct. 5, 1917; thereafter absent on 500 gm. of lung and 100 gm. of suet, with development of hyperglycemia. Intravenous glucose injections, 1.3 gm. per kg. per hr. in 10 per cent solution (4 injections per hr.), based on 15 kg. weight.

Time.	Period A. Aug. 6, 1917. Weight 16.2 kg.			Period B. Dec. 4, 1917. Weight 14.2 kg.			Period C. Feb. 19, 1918. Weight 15.5 kg.		
	Urine.		Plasma sugar.	Urine.		Plasma sugar.	Urine.		Plasma sugar.
	Volume.	Glucose.		Volume.	Glucose.		Volume.	Glucose.	
	cc.	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*
Before injection		0	0.106		0	0.104		0	0.154
End 1st hr.	20	1.56	0.278	100	2.84	0.715	46	4.83	0.625
" 2nd "	30	2.87	0.294	144	4.45	0.990	214	4.00	0.800
" 3rd "	64	1.71	0.312	222	3.65	0.990	252	4.35	1.000
" 4th "	106	0.76	0.270	300	2.80	1.000	274	3.70	1.180
" 5th "	110	0.42	0.218	220	2.93	0.844	281	4.25	1.110
" 6th "	76	0.53	0.204	265	2.43	0.844	270	4.55	1.050
" 7th "	128	0.51	0.183	222	2.71	0.625	222	4.55	1.000
" 8th "				216	3.82	0.715	278	5.13	0.950
1 hr. after injection	90	Faint.	0.082	60	3.60	0.370	78	5.88	0.525
2 hrs. " "	82	0	0.098	66	0.49	0.217	10	5.13	0.322

A. Total solution injected 1,560 cc.
" glucose " 156 gm.
" urine excreted. 706 cc.
" glucose " .. 4.5 gm.
Per cent of dose..... 2.87 per cent.

B. Total solution injected 1,560 cc.
" glucose " . 156 gm.
" urine excreted. 805 cc.
" glucose " . 55.2 gm.
Per cent of dose..... 35.4 per cent.

C. Total solution injected 1,560 cc.
" glucose " . 156 gm.
" urine excreted. 1,925 cc.
" glucose " . 80.4 gm.
Per cent of dose..... 51.5 per cent.

TABLE XXXII.

Percentage of Total Intravenously Injected Dose of Glucose Excreted by Normal Dogs.

Dosage per kg. per hr.	1 gm. in 10 per cent solution (3 injections per hr.).	1 gm. in 20 per cent solution (3 injections per hr.).			1 gm. in 5 per cent solution (2 injections per hr.).	1.5 gm. in 20 per cent solution (3 injections per hr.).							1.5 gm. in 10 per cent solution (4 injections per hr.).	1.5 gm. in 7.5 per cent solution (2 injections per hr.).	2 gm. in 5 per cent solution (continuously).
Duration	For 2 hrs.	For 3 hrs.	For 10 hrs.	For 3 hrs.	For 2 hrs.	For 2 hrs.		For 3 hrs.					For 4 hrs.	For 9 hrs.	For 2 hrs.	For 4 hrs.
Table No.	I	II	III	VIII	IV (A)	V (A)	VI	V (B)	VII	IX	X	XI	XII	XIII	IV (B)	XIV
Per cent of total dose excreted	0.73	2.16	1.48	8.40	0	2.28	4.40	1.41	1.60	1.50	1.50	1.28	1.40	1.88	2.27	5.90
Per cent of total dose excreted for first 2 hrs.			0.85											Trace.		
Per cent of total dose excreted for first 3 hrs.			1.40											0.96		

TABLE XXXIII.

Percentage of Total Dose of Intravenously Injected Glucose Excreted by Partially Depancreatized Dogs.

	No diabetes.		Very mild diabetes.			Mild diabetes.			Moderate diabetes.			Severe diabetes.		
Table No..	XV	XVI	XVII (A)	XVIII	XIX (A)	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII	XXVIII (A)
Dosage per kg. per hr.	1 gm. in 20 per cent solution (3 injections per hr.).	1.5 gm. in 5 per cent solution (3 injections per hr.).	1.2 gm. in 5 per cent solution (continuous).	1.5 gm. in 5 per cent solution (3 injections per hr.).	1 gm. in 20 per cent solution (3 injections per hr.).	1 gm. in 5 per cent solution (3 injections per hr.).	1 gm. in 20 per cent solution (3 injections per hr.).	1 gm. in 20 per cent solution (3 injections per hr.).	1 gm. in 20 per cent solution (4 injections per hr.).	1 gm. in 20 per cent solution (3 injections per hr.).	1 gm. in 10 per cent solution (4 injections per hr.).	1 gm. in 5 per cent solution (4 injections per hr.).	1.5 gm. in 5 per cent solution (3 injections per hr.).	1 gm. in 20 per cent solution (3 injections per hr.).
Duration.	For 3 hrs.	For 2 hrs.	For 3 hrs.	For 1 hr.	For 2 hrs.	For $3\frac{1}{2}$ hrs.	For 2 hrs.	For 3 hrs.	For 2 hrs.	For $2\frac{1}{3}$ hrs.	For 2 hrs.	For $2\frac{1}{4}$ hrs.	For 1 hr.	For 2 hrs.
Per cent of total dose excreted.	2.2	5.6	14.1	10.5	3.02	0	8.2	26.4	20.4	19.8	22.2	20.5	23.3	1.69

ALKALI RESERVE OF SWINE AS AFFECTED BY CEREAL FEEDING AND MINERAL SUPPLEMENTS.

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It is common knowledge among students of animal nutrition that cereal rations are characterized by excess of acid as compared with basic mineral elements, and by a marked deficiency of calcium. A question remains, however, as to whether this potential acidity of cereal rations is of such degree as to constitute it a practical consideration, and especially as to the extent to which this acidity should be considered as merely incidental to the calcium shortage.

In the investigation of the physiological significance of the potential acidity of cereal rations it becomes a matter of much interest to determine the influence of this factor in relation to the composition and functional efficiency of the blood and tissues. A significant and fundamental matter in this connection is the maintenance of the alkali reserve, which may be considered, in general terms, as composed of those constituents of the body which can be used for acid neutralization. In the method of estimation of Van Slyke, Stillman, and Cullen,[1] which was used in this study, the alkali reserve is determined as the bicarbonate concentration of the blood plasma.

Ammonia and acidity were estimated by the methods of Folin, and hydrogen ion concentration by the colorimetric method of Clark and Lubs, using the colored screen and the comparator.

The blood samples were taken from the tail, the handling of the subjects being successfully accomplished by the use of a crate devised for hog-cholera serum work. The tail was shaved, the

[1] Van Slyke, D. D., Stillman, E., and Cullen, G. E., *J. Biol. Chem.*, 1919, xxxviii, 167.

end clipped off, and the blood drawn through a 5 cm. funnel into a 50 cc. centrifuge tube where it was collected under a 0.5 cm. layer of liquid petrolatum (Squibb). The funnel was fitted to the tube with a two-hole rubber stopper, and the funnel and tube together contained powdered potassium oxalate equal to 0.1 per cent of the blood drawn. The tail was held inside the funnel, with the bleeding tip within 1 or 2 cm. of the bottom, to minimize possible loss of carbon dioxide.

In this study two pigs were fed a cereal ration composed of corn 7 parts, wheat middlings 1 part, and linseed oil meal 1 part, with salt (NaCl) weighed separately at a rate of 2.2 gm. per kilo of feed. The two pigs weighed 65.0 and 62.4 kilos, respectively, on October 14, at the beginning of the experimental treatment, and on January 2, 112.3 and 110.0 kilos, respectively. The experiment terminated on January 3. The pigs were fed in metabolism crates, and cared for as in metabolism experiments.

In the first period the cereal ration was fed alone for 33 days, during the last 7 of which alkali reserve estimations on the blood plasma were made twice on each subject.

In the second period there was then added to the cereal ration calcium carbonate in amounts equivalent to 200 mg. of calcium per kilo of live weight of the pigs. This treatment was continued for 25 days, during the last 12 of which alkali reserve estimations were made on the two subjects three and four times, respectively, at intervals as shown in Table I.

In the third period the mineral supplement was changed to precipitated bone phosphate, which is largely in the dicalcic form, and which was fed in amounts furnishing, as before, 200 mg. of calcium per kilo of live weight. This treatment was continued for 23 days, during the last 13 of which alkali reserve estimations were made four times on each subject. The condensed data of the experiment will be found in Table I. Alkali reserve estimations were made on the days indicated, while estimations of urinary acid, ammonia, and hydrogen ion concentration were made daily during the greater part of the study and on alternate days during the remainder.

The data show that by the addition of calcium carbonate to a cereal ration the alkali reserve of the blood plasma was increased 10.1 and 10.8 per cent, with the two pigs, above that which pre-

TABLE I.

Effects of Cereal Ration and Mineral Supplements on Blood Plasma and Urine of Swine.

Pig No.	Treatment.	Date of estimation.	Blood plasma.		24 hr. urine.		
			Molecular concentration of CO_2 as bicarbonate.	CO_2 as bicarbonate.	Acidity.	Ammonia.	H ion concentration.
		1919		*vol. per cent*	*cc. 0.1 N*	*cc. 0.1 N*	*pH*
1	Basal ration of cereals	Nov. 11	0.0297	66.5			
1	Basal ration of cereals	" 17	0.0314	70.3			
Average			0.0306	68.4	255 (8)*	4.18 (8)	7.1 (8)
1	Calcium carbonate	Nov. 29	0.0338	75.7			
1	" "	Dec. 3	0.0354	79.3			
1	" "	" 9	0.0327	73.2			
1	" "	" 11	0.0328	73.5			
Average			0.0337	75.4	—49 (13)	1.71 (13)	7.7 (13)
1	Precipitated bone phosphate	Dec. 22	0.0269	60.3			
1	Precipitated bone phosphate	" 26	0.0282	63.2			
		1920					
1	Precipitated bone phosphate	Jan. 2	0.0295	66.1			
1	Precipitated bone phosphate	" 3	0.0303	67.9			
Average			0.0287	64.4	705 (15)	4.01 (15)	6.36 (15)
		1919					
2	Basal ration of cereals	Nov. 12	0.0323	72.3			
2	Basal ration of cereals	" 17	0.0323	72.4			
Average			0.0323	72.4	638 (8)	4.10 (8)	6.5 (8)

* The numbers in parentheses indicate the number of daily estimations averaged.

TABLE I—*Concluded.*

Pig No.	Treatment.	Date of estimation.	Blood plasma.		24 hr. urine.		
			Molecular concentration of CO_2 as bicarbonate.	CO_2 as bicarbonate.	Acidity.	Ammonia.	H ion concentration.
		1919		*vol. per cent*	*cc. 0.1 N*	*cc. 0.1 N*	*pH*
2	Calcium carbonate...	Nov. 29	0.0350	78.4			
2	" " ...	Dec. 3	0.0355	79.5			
2	" " ...	" 9	0.0370	82.9			
Average.....			0.0358	80.3	−29 (13)	1.06 (13)	7.6 (13)
2	Precipitated bone phosphate.....	Dec. 22	0.0306	68.5			
2	Precipitated bone phosphate.....	" 26	0.0314	70.3			
		1920					
2	Precipitated bone phosphate.....	Jan. 2	0.0281	62.9			
2	Precipitated bone phosphate.....	" 3	0.0313	70.1			
Average.....			0.0303	68.0	1,707 (15)	3.89 (15)	5.57 (15)

vailed during the feeding of the cereal ration alone; and that then, by the substitution of precipitated bone phosphate for the calcium carbonate, the alkali reserve was reduced 14.8 and 15.4 per cent, with the two subjects, to figures which are distinctly lower, in each case, than those obtained from the same individual on the cereal ration. The estimations of acidity, ammonia, and hydrogen ion concentration in the urine vary, as affected by the mineral supplements, in a manner concordant with the alkali reserve estimations, which they serve to confirm. The separate estimations of urinary acidity reveal marked daily and enormous individual variation in the details of the eliminative function.

The alkali reserve of the blood plasma of swine, therefore, is susceptible of variation, and may be either increased or decreased by the use of mineral supplements added to a cereal ration in such quantities as might be used in practical feeding. Since

these variations would in all probability be followed, in time, by variations in the other alkali reserves of the body, it is more than likely that changes of body function would result. These might be favorable or unfavorable in accord with the ration and mineral supplements used.

In this connection we are unable to follow Lamb and Evvard[2] in some of the conclusions which they draw from their study of the acid-base balance in swine. Thus, contrary to their main conclusion, "On neither ration did the mineral acid cause a significant loss of calcium, nor did it interfere with the storage of protein," their data for Experiment 1 show, as a result of mineral acid ingestion, a decreased retention of calcium amounting to one-fourth or one-fifth according to whether it is computed with reference to the *absolute amount* or the *proportion of the intake* retained. It should also be noted that the results from Experiment 2 are conflicting since the first control period makes it appear that the mineral acid feeding had *diminished* the loss of calcium by 0.030 gm. of CaO per day, while the second or following control period indicated, with opposite significance, that the acid feeding had *increased* the loss by 0.550 gm. of CaO per day. The authors averaged these contrary findings.

Further, their finding that the mineral acid ingestion had not affected the hydrogen ion concentration of the blood was beside the point, since this constant would increase only as death was about to supervene; and their evidence as to the effect of acid ingestion on the reproductive activity of the sows lacks conclusiveness since only one pig was raised out of eleven farrowed.

We believe therefore that the ultimate effects of the potential acidity of cereal rations on the functional activities of swine remain yet to be determined.

CONCLUSION.

The alkali reserve of the blood plasma of swine may be significantly increased by the feeding of the potentially basic precipitated calcium carbonate, or decreased by the feeding of the potentially acid precipitated calcium phosphate, when these substances are fed as supplements to a cereal ration in quantities such as might be used in practical feeding.

[2] Lamb, A. R., and Evvard, J. M., *J. Biol. Chem.*, 1919, xxxvii, 317.

THE OCCURRENCE OF WATER-SOLUBLE VITAMINE IN SOME COMMON FRUITS.*

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Despite the fact that the edible fruits enter liberally into the dietary of man in nearly every part of the world, they have hitherto received almost no serious consideration at the hands of scientific investigators. Usually they are casually grouped for consideration with the familiar vegetables with which, it may be noted, they have many features of composition in common. Like these vegetables most fruits contain considerable water; some of them furnish a liberal amount of sugar or starch; and they cannot be classed as significant sources of fat or protein. One may read in government leaflets that

"fruits and vegetables are necessary for health because they supply certain needed substances that are not usually found in any other food materials. They should have a place in the diet of all those who have passed babyhood and no pains should be spared to obtain at least a small amount of them every day."[1]

The reason for such advice is found in the statement that

"fruits and vegetables are used in the diet to give pleasant flavor and varied texture. They are important not only for this but because they give bulk and are laxative; because they contain valuable mineral salts, such as

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, Washington, D. C.

[1] The day's food in war and peace, U. S. Food Administration, U. S. Dept. Agric. leaflet, 75.

lime and iron; and because they furnish the dietary essentials sometimes called vitamines."[2]

The explanation of the asserted laxative action of the fruits is sought in part in their content of "certain mild vegetable acids useful in preventing constipation." A recent review summarizes the status of fruits in these words:

"No thorough studies of the dietary properties of fruits have yet been made, but from their known chemical composition and biological functions as storage organs, their proper place in the diet can be predicted. They are good sources of mineral salts and of energy-yielding foods, the sugars. They are highly palatable and exert a favorable influence on the excretory processes of the kidneys and the intestine. Their liberal use in the diet should be encouraged."[3]

In so far as the content of inorganic constituents is concerned it must be noted that the edible portions of the fruits and the quantities in which they are customarily eaten in general furnish comparatively little of those elements, notably calcium and phosphorus, which alone deserve more serious consideration in the selection of the dietary.[4] Hence they merit less attention from the standpoint of their "mineral nutrients." It has long been appreciated, however, that some of the fruits have a potency, formerly quite inexplicable, in preventing the onset of scurvy under the conditions now known to permit its appearance. Since the demonstration that this malady is to be classed with the deficiency diseases, the rôle of fruits as antiscorbutics has been widely investigated. The potency of the juice and inner peel of the orange, the juice of the lemon, and some of the rarer fruits, such as the tamarind, cocum, and mango,[5] has been established experimentally on animals and man. On the other hand the antiscorbutic value of the West Indian lime, *Citrus medica acida*, long cherished by the public, has been demonstrated to be insignificant in comparison with the efficacy of other citrus fruits, *Citrus medica limonum*.[6]

[2] The day's food in war and peace,[1] p. 76.

[3] McCollum, E. V., The newer knowledge of nutrition, New York, 1919, 142.

[4] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxiv, 131.

[5] Chick, H., Hume, E. M., and Skelton, R. F., *Lancet*, 1919, ii, 320.

[6] Chick, H., Hume, E. M., and Skelton, R. F., *Lancet*, 1918, ii, 735.

According to Lewis[7] the banana contains some antiscorbutic vitamine. Hess and Unger[8] concluded from their investigations on scorbutic infants that "the banana possesses but mild antiscorbutic virtue" when compared to other commonly used fruits.

With respect to the distribution of the other vitamines—the so called fat-soluble A and water-soluble B—now recognized as physiologically distinct accessory food factors, almost no experimental data are available. In the case of the banana Loeb and Northrop[9] have found that under aseptic conditions the fruit fly, *Drosophila ampelophila*, cannot be raised upon bananas, but will grow when yeast is supplied in addition. This suggests the possibility of a deficiency of the banana in the water-soluble vitamine—a conclusion also reached by Sugiura and Benedict[10] from experiments on rats.

Harden and Zilva[11] have concluded, as a result of a study of the action of adsorbents upon autolyzed yeast and orange juice (representing sources of antineuritic and antiscorbutic vitamine, respectively) and mixtures of them, that the two vitamines are not identical, because the potencies characteristic of them could be separated by fullers' earth or dialyzed iron. Incidentally these investigators state: "we have so far not come across a natural product which contains both [antineuritic and antiscorbutic factors] in quantities suitable for investigation" (p. 100). The implication thus remains that orange juice exhibits only the antiscorbutic quality in noteworthy amounts.[12]

[7] Lewis, H. B., *J. Biol. Chem.*, 1919, xl, 91.

[8] Hess, A. F., and Unger, L. J., *Am. J. Dis. Child.*, 1919, xvii, 221.

[9] Loeb, J., and Northrop, J. H., *J. Biol. Chem.*, 1916, xxvii, 309. Northrop, J. H., *J. Biol. Chem.*, 1917, xxx, 181.

[10] Sugiura, K., and Benedict, S. R., *J. Biol. Chem.*, 1918, xxxvi, 171.

[11] Harden, A., and Zilva, S. S., *Biochem. J.*, 1918, xii, 93.

[12] In a paper which has appeared since this manuscript was prepared, Byfield, Daniels, and Loughlin have reported that in experiments on babies and on rats "growth, as evidenced by the weight curves, was in all cases stimulated when orange juice was given. On the other hand, orange juice from which the antineuritic vitamin has been removed was without influence." To these investigators "it appears that the appetite factor plays only a minor rôle in the stimulating effect of the water soluble vitamin on growth, provided a nearly adequate amount is being given." They also conclude that "Orange juice from which the antineuritic vitamin is

Experiments with Fruits as Sources of Vitamines.[13]

Methods of Feeding.—The routine customary in our laboratory in nutrition experiments on albino rats has been followed in this investigation of the fruits. The standard food mixture consisted of

	per cent
Meat residue	19.6
Salt mixture*	4.0
Starch	52.4
Butter fat	9.0
Lard	15.0

*For composition of salt mixture see Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 557.

This was selected, in accord with earlier experience,[14] to furnish a diet free from water-soluble vitamine (B). The possible presence of the latter in the various fruits was tested for by furnishing daily a measured amount of the fresh fruit, the fruit juice, or dried products thereof apart from the rest of the ration. In a few cases the addendum was incorporated in the food mixture, but as a rule it was supplied separately so as not to alter the flavor or palatability of the standard diet. The effects of the presence or removal of the fruit products on nutrition as expressed in food intake and body weight are shown in selected tables and charts in the appendix.

Orange.—The fresh juice, prepared by squeezing the skinned oranges, *i.e.* the edible portion,[15] contains sufficient water-soluble vitamine to promote growth in rats at about the same rate as do

removed by adsorption does not stimulate growth. This would seem to indicate that the antiscorbutic vitamin lacks growth-stimulating properties" (Byfield, A. H., Daniels, A. L., and Loughlin, R., *Am. J. Dis. Child.*, 1920, xix, 349).

[13] A preliminary notice of some of the results obtained was published by Osborne, T. B., and Mendel, L. B., *Proc. Soc. Exp. Biol. and Med.*, 1919, xvii, 46.

[14] Osborne, T. B., Wakeman, A. J., and Ferry, E. L., *J. Biol. Chem.*, 1919, xxxix, 35.

[15] Data regarding this are given by Joslin, E. P., The treatment of diabetes mellitus, Philadelphia and New York, 2nd edition, 1917, 265.

the same volumes of milk.[16] This is apparent from Charts I and II in which the effects of varying daily doses (2.5 cc., 5 cc., 10 cc.) of orange juice as the source of water-soluble vitamine upon the growth of rats are shown. In our similar experiments with cow's milk a daily intake of at least 10 to 15 cc. was needed to produce growth at a rate approaching the average. Illustrative data regarding food intakes in the orange experiments are summarized in Table I. The fall in body weight accompanying the decline in food intake when the feeding of orange juice was discontinued is indicated in Chart I (Rats 6106, 6127, 6141) and in Chart II (Rats 6333, 6338), as well as the characteristic restoration following renewed administration of this fruit product. That the advantage of the latter is attributable merely to increased calories (sugar, fruit acids, etc.), *i.e.* to supplementing the basal diet, is not a tenable assumption.

Orange juice was desiccated by preliminary concentration *in vacuo* and drying upon starch in a current of warm air. This dry product,[17] fed in quantities equivalent to measured portions of fresh orange juice, was apparently of equal potency as a source of water-soluble vitamine (B) (Chart II). Givens and McClugage[18] have shown that the antiscorbutic properties of orange juice are likewise not lost by suitable desiccation.

Inasmuch as it has been found by Hess and his associates[19] that the inner peel of the orange has considerable antiscorbutic potency we tested this product both fresh and dried for water-soluble B. When the rats would eat the peel, doses represent-

[16] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxiv, 537; *J. Biol. Chem.*, 1920, xli, 515. In their recently published observations upon the growth-promoting effects of orange juice on rats, Byfield, Daniels, and Loughlin[12] showed that, with a ration essentially like our standard diet, 55 cc. of orange juice per 100 gm. of food were insufficient to promote maximal growth, 75 cc. per 100 gm. being more efficacious. Our own data are given in Table I.

[17] We have lately fed such materials in the form of tablets compressed with a machine which secures accurate regulation of dosage.

[18] Givens, M. H., and McClugage, H. B., *Am. J. Dis. Child.*, 1919, xviii, 30.

[19] Hess, A. F., and Fish, M., *Am. J. Dis. Child.*, 1914, viii, 385. Hess, A. F., and Unger, L. J., *J. Biol. Chem.*, 1918, xxxv, 487. Hess, A. F., *J. Am. Med. Assn.*, 1918, lxxi, 941.

ing 5 gm. per day were quite efficacious (Chart III). The failure to grow vigorously, which is shown during the earlier periods in the records of Rats 6134, 6137, and 6138, is associated with the refusal to consume entirely the portions of peel offered to them daily.

Lemon.[15]—The procedure with lemon juice was essentially like that for the orange, dried products being tested. The quantitative relations are about the same, quantities less than 10 cc. of juice per day being insufficient as sources of the water-soluble vitamine for growing rats (Chart IV).

Grapefruit.[15]—Desiccated juice prepared from the edible portion was used in quantities equivalent to 10 cc. of the fresh juice (Chart V). It contains water-soluble vitamine.

Grape.—A commercial grape juice (Welch's) used in 10 cc. doses of the fluid or its equivalent desiccated on starch was tested (Chart VI). Compared volume for volume these products were far less potent than orange, lemon, or grapefruit juices.

Apple and Pear.—In the case of these fruits their bulky character made it practically impossible to feed to a rat more than 10 gm. of the fresh product per day without a reduction of the essential basal ration to a quantity incompatible with maintenance. That some water-soluble vitamine is furnished by apples and pears is shown by the rapid declines when these are withdrawn from the otherwise adequate diet (Charts VII and VIII). In several cases the superior potency of 0.1 to 0.2 gm. of dried brewery yeast per day is shown by contrast. A few preliminary tests with the juice expressed from 10 gm. of apple showed no advantage over the more bulky fresh fruit (Chart VII).

Prune.—Tests with prunes showed that, fed in 2 and 5 gm. daily portions, they are not devoid of water-soluble vitamine (B); the gains made were decidedly greater than those exhibited in the experiments with apples and pears (Chart IX).

Do the Fruits Contain the Fat-Soluble Vitamine?

Our experiments to secure an answer to this question are not yet completed. The indications are, however, that the juices of the lemon and grapefruit respectively, fed in the equivalent of 10 cc. daily doses, do not contain sufficient fat-soluble vitamine

to avert the decline in body weight or to heal the eye disease (xerophthalmia, keratomalacia) which may ensue when animals are kept for some time on a diet devoid of the fat-soluble factor. Since as little as 0.1 gm. of butter fat per day may suffice to restore these animals to health it seems unlikely at the present stage of our investigation that the juices of these fruits contain more than traces, if any, of the fat-soluble vitamine. In the case of the orange, preliminary tests indicate that a supply of dried juice has averted or at least delayed the symptoms just referred to as characteristic of a régime lacking fat-soluble vitamine. The observations are as yet too limited in number to permit a final decision on this point.

SUMMARY.

The fresh juices of the edible parts of the *orange*, *lemon*, and *grapefruit* contain water-soluble (B) vitamine. Their potency in this respect is quite similar to that of comparable volumes of cow's milk. The efficiency of these fruit juices is not lost by suitable modes of desiccation. A sample of *grape* juice tested was less potent than equal volumes of the fruit juices just mentioned.

The edible portions of *apples* and *pears* furnish some water-soluble vitamine; the quantity of these fruits necessary to supply this dietary essential is relatively very large, so that from a comparative standpoint they cannot be regarded as rich in this food factor. *Prunes* apparently are richer in the water-soluble vitamine.

From preliminary experiments it seems doubtful whether the juices of the lemon or grapefruit contain more than traces, if any, of the fat-soluble vitamine. Our preliminary observations upon orange juice are indicative of some potency in this vitamine.

The experiments with fruits place the dietary value of these foods, hitherto recommended because of their salt content, their laxative properties, or their antiscorbutic potency, in a new light as sources of water-soluble vitamine.

EXPLANATION OF TABLES.

The data here summarized indicate the successive weekly intakes (in gm.) of the basal ration by the rats for which the changes in body weight are represented graphically in the appended charts. The daily dose of the various fruit products (in equivalent cc. or gm.) is shown in boldfaced type at the beginning of each period. The figures may be studied in connection with the corresponding charts.

TABLE I.

Orange Juice (Charts I and II).

Rat 6141♂.	Rat 6127♂.	Rat 6106♂.	Rat 6256♀.	Rat 6338♂.	Rat 6333♂.	Rat 6427♂.	Rat 6428♂.	Rat 6375♂.	Rat 6373♂.	Rat 6372♂.
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
5 cc.	5 cc.	5 cc.	None.	10 cc.	10 cc.	5 cc.	5 cc.	5 cc.	5 cc.	5 cc.
53	57	64	33	32	35	44	46	31	47	35
(?)	(?)	(?)	28	44	40	44	37	42	47	43
10 cc.	10 cc.	10 cc.	(?)	52	41	21	19	36	2.5 cc.	2.5 cc.
42	49	48	5 cc.	62	47	42	39	29	41	29
39	50	46	44	53	47	50	30	27	37	30
47	56	50	50	59	46	28	26	30	28	25
51	48	46	45	49	48	33	33	22	20	24
51	50	46	41	None.	None.	37	37	18	27	24
54	47	51	33	38	38	37	36	23	28	21
(?)	54	56	40	21	37	10 cc.	10 cc.	28	28	27
65	45	54	51	(?)	26	57	44	26	28	20
60	54	54	39	5 cc.	5 cc.	65	54	23	22	24
63	59	59		40	31			10 cc.	17	24
56	54	57		27	29			34	15 mg.	15 mg.
48	54	49		31	29			20	yeast	yeast
None.	None.	None.		37	25			32	fraction.	fraction.
30	32	41		28	22				47	39
22	35	26		29	32				46	38
10 cc.	37	(?)		34	25				53	42
56	10 cc.	33		36	10 cc.					
67	56	29		10 cc.	34					
55	60	31		41	39					
	64	26		55	50					
	42	24								
	47	27								
	48	5 cc.								
	0.2	42								
	gm.	46								
	yeast.	41								
	75	45								
	65	36								
		39								
		34								
		42								
		None.								
		26								
		14								
		Dead.								

TABLE II.

Orange Inner Peel (Chart III).

Rat 6352♂.	Rat 6354♂.	Rat 6138♂.	Rat 6137♂.	Rat 6134♂.
gm.	*gm.*	*gm.*	*gm.*	*gm.*
5 gm.	**5 gm.**	**5 gm.**	**5 gm.**	**5 gm.**
(?)	(?)	54	60	51
48	37	53	54	45
55	43	45	48	38
42	37	36	37	33
47	34	44	44	37
37	32	37	56	42
32	33	40	55	39
31	36	40	47	40
29	51	45	51	53
16	46	44	51	52
36	48	46	39	46
37	48	64	47	49
34	58	69	**None.**	**None.**
25	52	70	34	22
30	56	62	(?)	19
33	57	41	**0.4 gm. yeast.**	Dead.
None.	**None.**	59	69	
27	31	64	82	
Dead.	24	58		
		58		

TABLE III.

Lemon Juice (Chart IV).

Rat 6325♂.	Rat 6324♂.	Rat 6329♂.	Rat 6441♂.	Rat 6442♂.
gm.	*gm.*	*gm.*	*gm.*	*gm.*
5 cc.	**5 cc.**	**5 cc.**	**2.5 cc.**	**2.5 cc.**
27	25	25	31	28
33	36	32	38	30
35	30	37	27	33
10 cc.	**10 cc.**	**10 cc.**	27	27
44	40	48	27	Dead.
57	35	(?)	27	
56	41	54	**10 cc.**	
46	40	52	29	
59	45	57		
None.	**None.**	**None.**		
42	39	(?)		
22	19	37		
22	15	(?)		
5 cc.	**5 cc.**	**5 cc.**		
44	40	40		
42	29	35		
46	34	30		
41	27	40		
39	35	37		
36	28	34		
40	34	31		
35	30	30		

TABLE IV.

Grapefruit (Chart V).

Rat 6396 ♂.	Rat 6399 ♂.	Rat 6394 ♂.
gm.	*gm.*	*gm.*
10 cc.	**10 cc.**	**10 cc.**
35	45	38
47	52	38
55	41	43
58	50	45
49	38	45
50	44	31
48	43	23
42	33	31
36	42	33
35	38	32
33	34	17
51	**None.**	34
60	28	35
56	30	38
	36	

TABLE V.

Grape (Chart VI).

Rat 6381 ♂.	Rat 6377 ♂.	Rat 6380 ♂.	Rat 6336 ♀.	Rat 6337 ♀.
gm.	*gm.*	*gm.*	*gm.*	*gm.*
10 cc.	**10 cc.**	**10 cc.**	**None.**	**None.**
38	41	37	29	29
33	32	41	32	33
43	46	43	20	18
35	33	25	**10 cc.**	**10 cc.**
35	39	27	20	24
35	33	30	25	18
31	26	29	23	32
35	40	23	22	28
34	45	30	28	26
28	40	33	29	20
27	29	29	29	21
30	33	23	31	33
None.	35	**None.**	29	25
(?)	38	24	24	26
(?)		22	37	18
(?)		27	29	26
			21	22
			31	28
			None.	**None.**
			18	17
			20	22
			Dead.	Dead.

TABLE VI.

Apple (Chart VII).

Rat 5986♂.	Rat 5946♂.	Rat 5974♂.	Rat 5971♂.	Rat 6135♂.	Rat 6129♂.	Rat 6133♂.	Rat 6340♂.	Rat 6339♂.
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
4 gm.	**None.**	**None.**	**None.**	**5 gm.**	**5 gm.**	**5 gm.**	**5 cc.**	**5 cc.**
57	(?)	38	58	(?)	(?)	(?)	30	22
56	43	(?)	30	40	41	39	39	29
42	26	**4 gm.**	(?)	31	38	28	34	28
10 gm.	**4 gm.**	38	**4 gm.**	**10 gm.**	(?)	**10 gm.**	26	28
31	24	**8 gm.**	20	29	**10 gm.**	20	22	25
43	(?)	34	**8 gm.**	40	36	27	(?)	**10 cc.**
35	**0.2 gm.**	36	35	28	28	24	**10 cc.**	29
32	**yeast.**	**10 gm.**	32	38	37	28	22	20
41	51	34	**10 gm.**	32	25	26	**0.1 gm.**	**0.1 gm.**
39	(?)	45	28	33	28	24	**yeast.**	**yeast.**
34	**10 gm.**	41	32	29	41	19	31	44
36	45	39	34	36	37	Dead.	41	43
38	50	37	29	**None.**	**None.**		49	39
33	37	31	31	24	Dead.			
0.2 gm.	34	28	23	Dead.				
yeast.	31	36	27					
79	31	33	27					
54	31	34	30					
53	51	**0.2 gm.**	29					
	37	**yeast.**	**0.2 gm.**					
	36	73	**yeast.**					
	None.	62	71					
	49	66	51					
	22		50					
	(?)							
	0.2 gm.							
	yeast.							
	68							
	52							
	52							

TABLE VII.

Pear (Chart VIII).

Rat 6254♂.	Rat 6240♂.	Rat 6139♂.	Rat 6122♂.	Rat 6131♂.
gm.	*gm.*	*gm.*	*gm.*	*gm.*
10 gm.	**10 gm.**	**5 gm.**	**5 gm.**	**5 gm.**
35	31	(?)	(?)	(?)
45	34	34	49	37
46	36	29	33	27
36	32	29	**10 gm.**	24
41	39		33	20
34	37		27	32
22	37		27	30
0.2 gm. yeast.	**None.**		32	30
60	15		38	31
54	(?)		44	31
64	**0.2 gm. yeast.**		42	32
	39		44	29
	42		38	**10 gm.**
	44		31	25
			0.2 gm. yeast.	31
			83	32
			73	32
			67	**None.**
				25
				Dead.

TABLE VIII.

Prune (Chart IX).

Rat 6477♂.	Rat 6474♂.	Rat 6458♂.	Rat 6478♂.	Rat 6429♂.	Rat 6449♂.
gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
2 gm.	**2 gm.**	**2 gm.**	**5 gm.**	**5 gm.**	**5 gm.**
29	22	26	19	43	31
44	37	39	49	48	46
53	46	47	**1 gm.**	48	42
	38	38	51	27	33
	57	36		48	34
				53	34
				46	55
				48	
				41	
				2 gm.	
				34	
				48	

EXPLANATION OF CHARTS.

In the following charts showing changes in body weight the uninterrupted lines represent the periods during which the indicated quantities of the various fruits, estimated on the fresh basis, were fed daily. During the periods represented by the dotted line (- - - - -) no water-soluble vitamine whatever was fed. In a number of the experiments dried brewery yeast was used for comparison as a source of the water-soluble vitamine during the periods represented by the interrupted line (-·-·-·-). The rapid restoration of weight when fruits have been used as the source of water-soluble vitamine after a period of decline in body weight upon rations devoid of this food factor is characteristic of what we have previously termed the "curve of repair." Under these circumstances quantities of the fruits which are insufficient during the ordinary course of growth to promote the maximum increment of weight may suffice to secure rapid restoration in size.

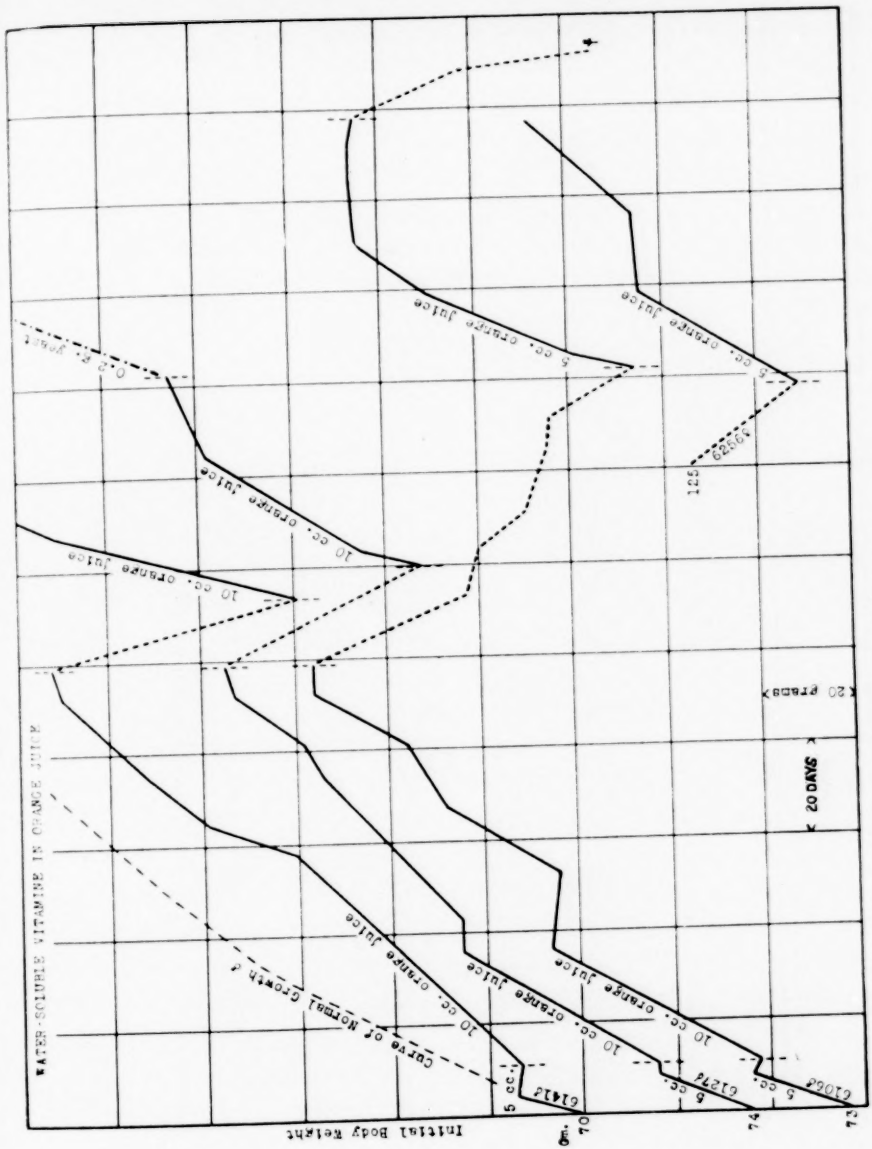
WATER-SOLUBLE VITAMINE IN ORANGE JUICE
Curve of Normal Growth
5 cc. orange juice
10 cc. orange juice
10 cc. orange juice
5 cc. orange juice
5 cc. orange juice
0.2 g. yeast
20 grams
20 DAYS
61062
61274
61412
5 cc.
Initial Body Weight
g.

CHART I.

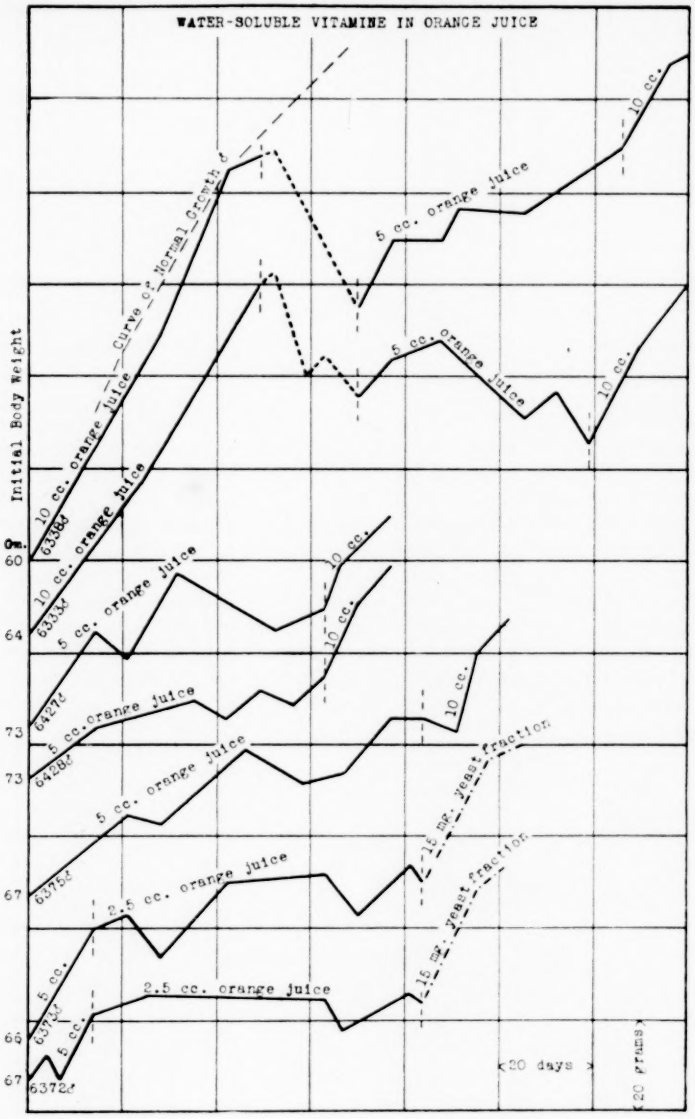
WATER-SOLUBLE VITAMINE IN ORANGE JUICE
Gm. Initial Body Weight
60
64
73
73
67
66
67
Curve of Normal Growth
10 cc. orange juice
5 cc. orange juice
10 cc.
63384
63384
64274
64282
63758
63792
63726
2.5 cc. orange juice
15 mg. yeast fraction
20 days
20 grams

CHART II.

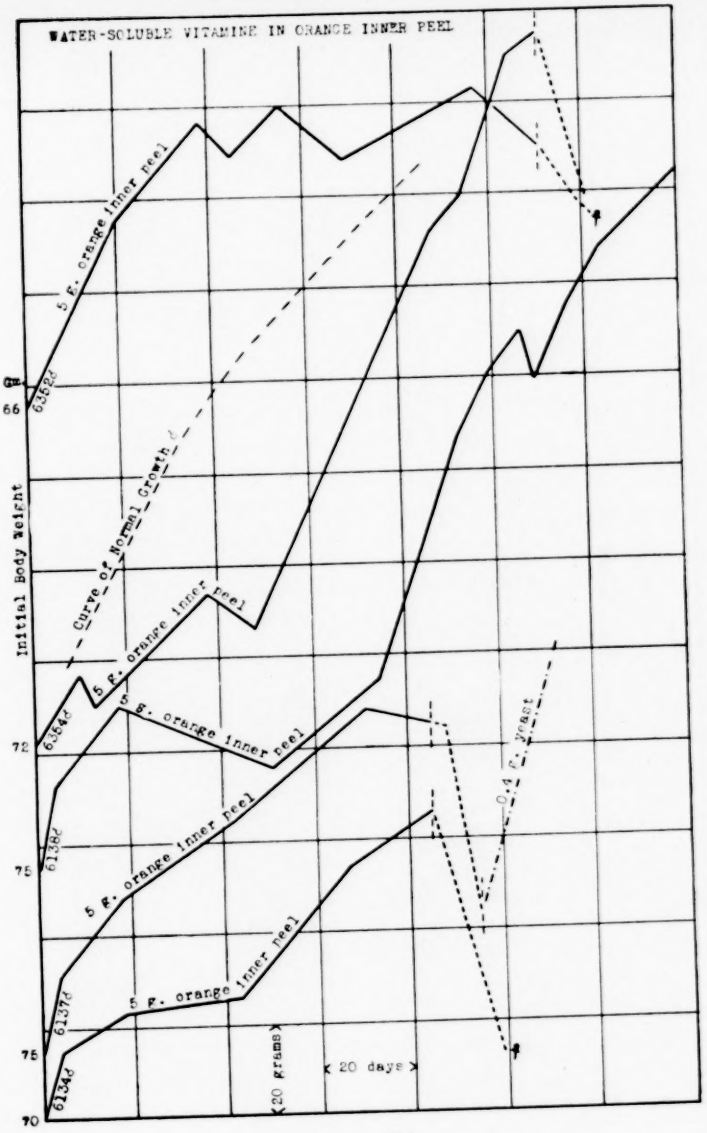
WATER-SOLUBLE VITAMINE IN ORANGE INNER PEEL
Initial Body Weight
Gm.
66
72
75
75
70
5 g. orange inner peel
6482d
Curve of Normal Growth
5 g. orange inner peel
6384d
5 g. orange inner peel
6138d
5 g. orange inner peel
6137d
5 g. orange inner peel
6134d
0.4 g. yeast
(20 grams)
20 days

CHART III.

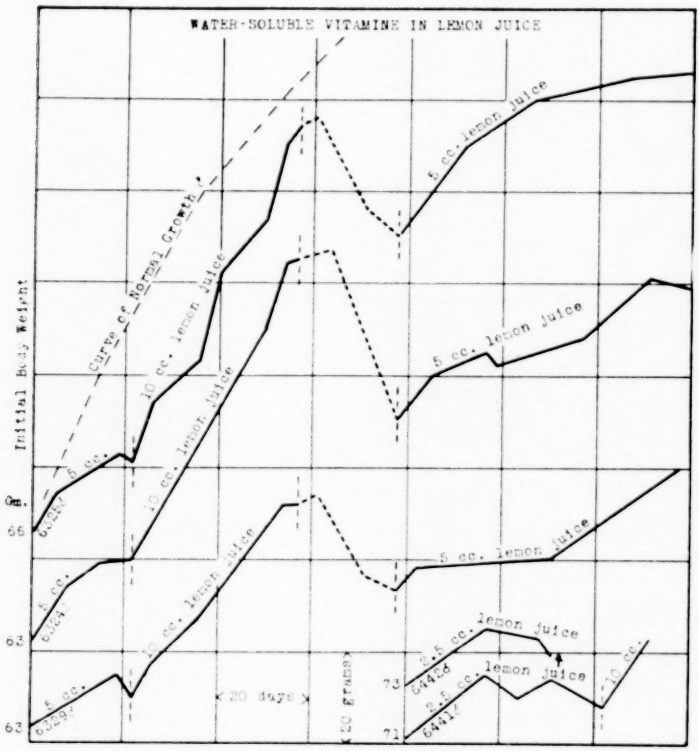
WATER-SOLUBLE VITAMINE IN LEMON JUICE
Curve of Normal Growth
Initial Body Weight
5 cc. lemon juice
10 cc. lemon juice
5 cc. lemon juice
10 cc. lemon juice
5 cc. lemon juice
5 cc. lemon juice
10 cc. lemon juice
5 cc. lemon juice
5 cc. lemon juice
5 cc. lemon juice
10 cc.
20 days
10 grams
66
63
63
73
71
64428
64417

Chart IV.

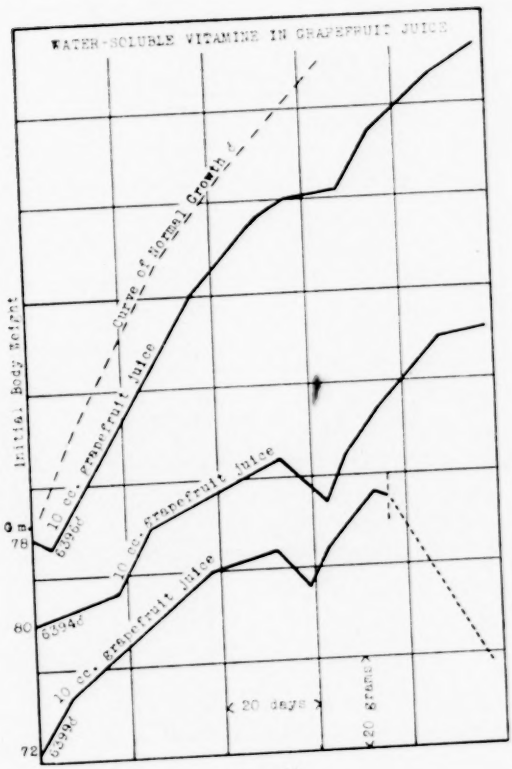
WATER-SOLUBLE VITAMINE IN GRAPEFRUIT JUICE
Initial Body Weight
Curve of normal Growth
10 cc. grapefruit juice
10 cc. grapefruit juice
10 cc. grapefruit juice
20 days
20 grams
78
80
72

Chart V.

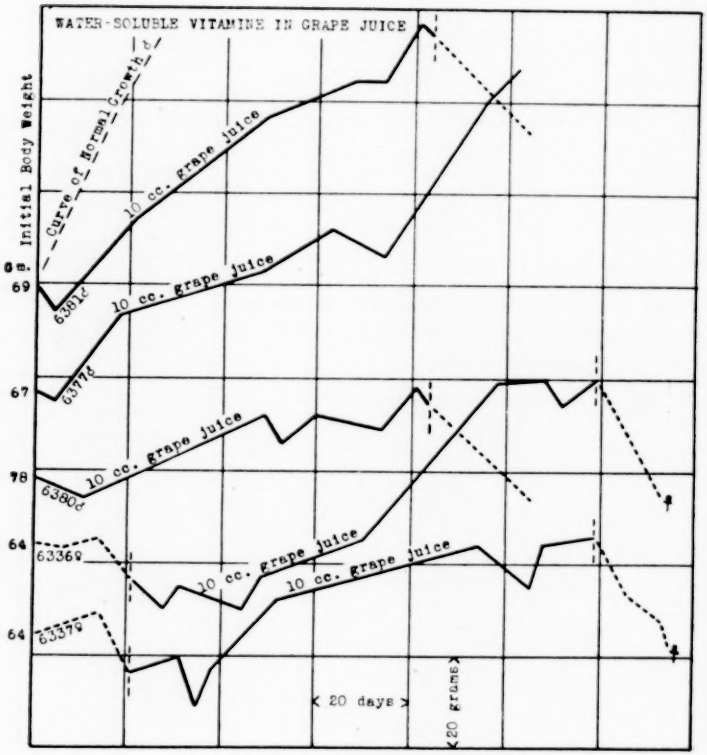
WATER-SOLUBLE VITAMINE IN GRAPE JUICE
Curve of Normal Growth
Gm. Initial Body Weight
69
67
78
64
64
10 cc. grape juice
10 cc. grape juice
10 cc. grape juice
10 cc. grape juice
10 cc. grape juice
63812
63778
63806
63369
63379
20 days
20 grams

CHART VI.

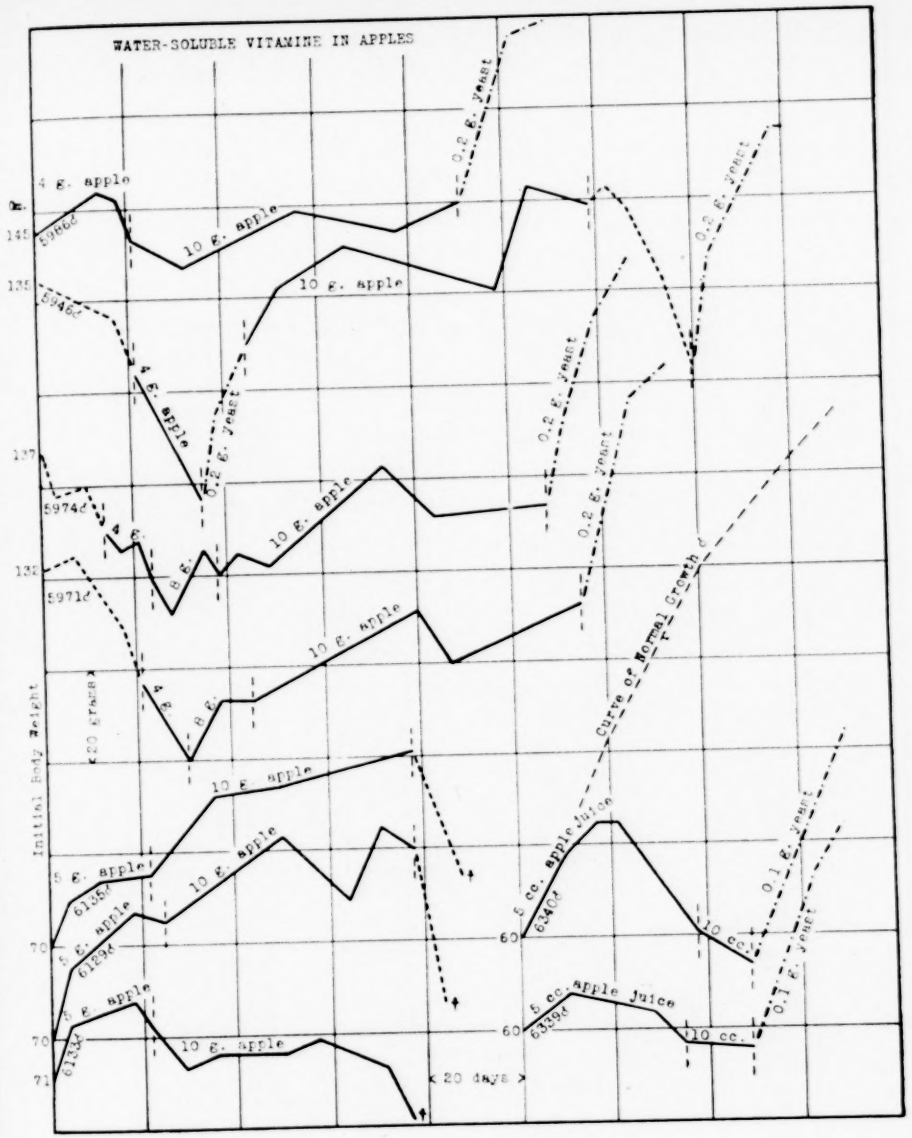
WATER-SOLUBLE VITAMINE IN APPLES
Initial Body Weight
4 g. apple
10 g. apple
0.2 g. yeast
5 cc. apple juice
10 cc.
0.1 g. yeast
Curve of Normal Growth
< 20 days >
< 20 grams

CHART VII.

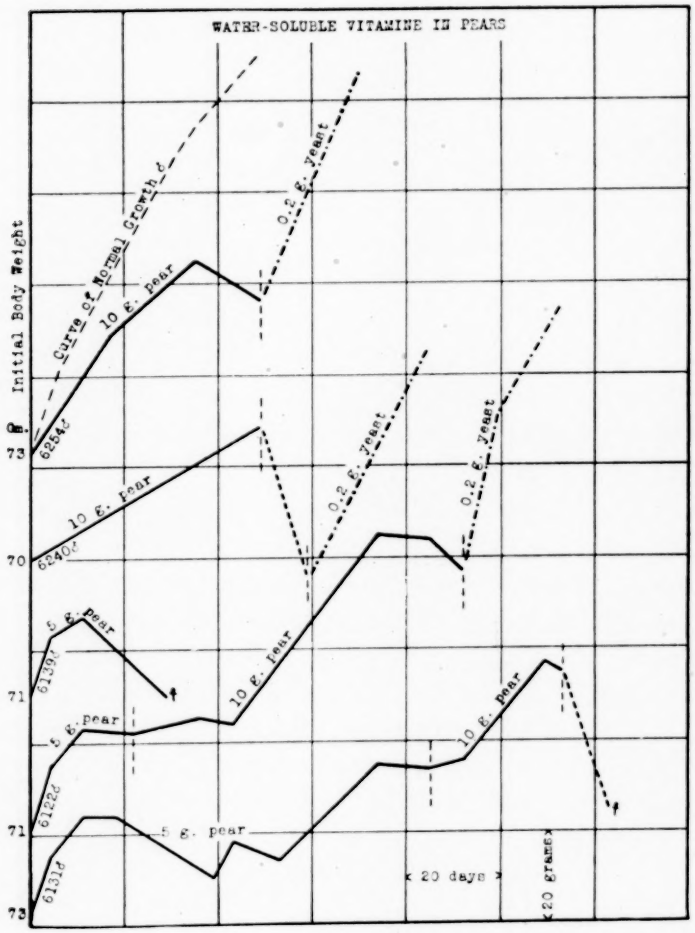
WATER-SOLUBLE VITAMINE IN PEARS
Initial Body Weight
Curve of Normal Growth ♂
10 E. pear
0.2 E. yeast
6284♂
10 E. pear
0.2 E. yeast
0.2 E. yeast
6240♂
5 E. pear
6139♂
10 E. pear
5 E. pear
6122♂
5 E. pear
10 E. pear
6131♂
20 days
20 grams

CHART VIII.

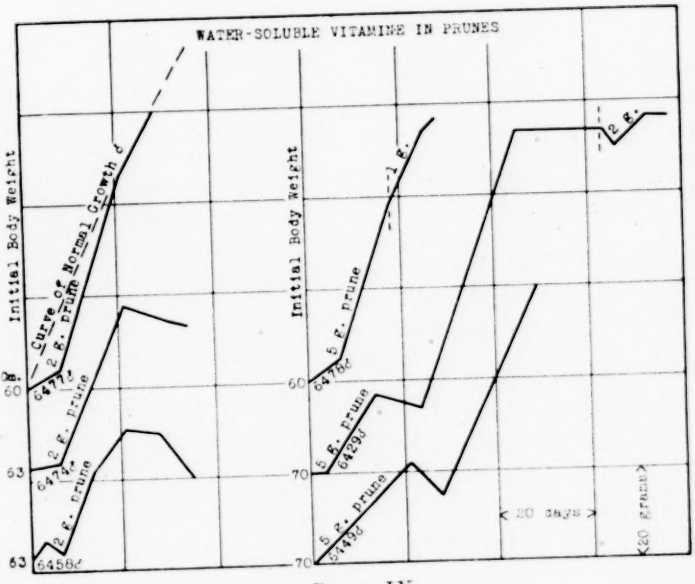
WATER-SOLUBLE VITAMINE IN PRUNES
Initial Body Weight
Curve of Normal Growth
2 F. Prune
64778
2 F. Prune
64774
2 F. Prune
64584
60
60
60
Initial Body Weight
1 F.
64782
5 F. Prune
64293
5 F. Prune
64436
2 F.
70
70
20 38/78
60 Prunes

CHART IX.

ANTISCORBUTIC PROPERTY OF VEGETABLES.

II. AN EXPERIMENTAL STUDY OF RAW AND DRIED POTATOES.

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The possibilities of preserving foods by desiccation are becoming recognized more and more. From some points of view drying is undoubtedly preferable to a great many other manipulations of foods to attain this end. However, such procedures should not be adopted without scientific proof that they do not affect adversely the foods dehydrated.

As part of a series of studies on the question whether or not the antiscorbutic vitamine in certain foods is destroyed at definite temperatures during drying we have already reported that under certain conditions cabbage (1), tomatoes (2), and orange juice (3) can be dehydrated so that the resultant dried products retain a significant amount of the antiscurvy vitamine. Data are reported herein concerning the antiscorbutic value of potatoes subjected to various temperatures and treatments.

The white potato is probably our most staple article of diet. Several investigations have been conducted to determine the value of its nitrogen-containing compounds (4-6). Biological experiments are available concerning its content of inorganic salts and water- and fat-soluble vitamines (7). The antiscorbutic property of the potato has been studied somewhat, but we are in need of more information upon this point (1, 8-10).

General Procedure and Methods.

Details of the general procedure and methods are described in the first paper of this series (1). The experimental animals were healthy young guinea pigs. The basal diet used to produce

scurvy was made of heated soy bean flour, milk, yeast, paper pulp, calcium lactate, and sodium chloride in the proportions hitherto reported. Tap water was offered to the animals *ad libitum*. Necropsies were performed as soon as possible after death. Scurvy was diagnosed on the basis of the usual clinical manifestations and postmortem findings. The occurrence of pneumonia in some of the animals is ascribed to the possibility that their general resistance was lowered owing to the minimal intake of antiscorbutic vitamine. Almost all the experiments later described are of the preventive type.

Potatoes were fed raw and cooked. The daily allotment of the undried samples was 10 gm. per animal. The dried potatoes or potatoes dried and cooked were fed to each animal in daily quantities of 2.5 gm. On this basis every animal received approximately the same amount of total solids of potatoes equivalent to that contained in 10 gm. of the raw material. When raw potatoes were cooked they were chopped very finely, boiling tap water was poured on them, and the water was kept boiling for 15 minutes. When the dried potatoes were cooked, the powdered material was suspended in a little cold water, poured into boiling water, and the whole boiled for 15 minutes. Potatoes baked and dried were fed as a paste.

Treatment of Potatoes.

In the study of potatoes subjected to different treatments, especial emphasis has been placed on a consideration of dried potatoes. The temperature of drying and the treatment previous to drying have varied. All potatoes dried, with the exception of those baked and dried, were first peeled. The precaution to slice the potatoes and remove the eyes was taken because the nitrogen content of the skin is higher than that of the body of the potato. At present we do not know to what extent the antiscorbutic vitamine may be associated with protein, particularly cellular material. The drying of all samples except Sample F was by means of hot air in a specially constructed apparatus (2).

The different ranges of temperature of drying were 35–40°, 55–60°, 75–80°, and baking at 204°, and then drying at 35–40°C. Potatoes dried at the first three ranges of temperature were

TABLE I.

Sample No.	Date dried.	Date started using.	Date finished using.	Duration of drying.
Potatoes dried at 35–40° C.				
	1918	*1918*	*1918*	*hrs.*
1	July 10	July 12	July 30	6–8
2	" 2	" 31	Aug. 12	6–8
3	" 3	Aug. 13	Sept. 16	6–8
4	" 2	Sept. 17	Oct. 14	6–8
81A	Oct. 10	Oct. 15	Nov. 2	6
81B	" 10	Nov. 3	" 23	6½
Potatoes dried at 55–60°C.				
7	July 10	July 12	July 29	7
16(2)	" 23	" 30	Aug. 12	6¾
19(1)	Aug. 1	Aug. 13	" 26	4
12	July 17	" 27	" 31	6¼
26(1)	Aug. 17	Sept. 1	Oct. 2	4¼
27(1, 2)	" 19	Oct. 3	Nov. 13	4½
Potatoes dried at 75–80°C.				
73(1, 2)	Oct. 7	Oct. 15	Dec. 4	3
			1919	
74(1, 2)	" 7	Dec. 5	Jan. 18	3⅛
	1919	*1919*		
189	Jan. 11	Jan. 19	" 29	2
190	" 11	" 30	Feb. 8	2½
Potatoes baked at 204°C. and dried at 35–40°C.				
	1918	*1918*	*1918*	
109(1, 2)	Oct. 25	Oct. 29	Nov. 26	7¼
			1919	
114(1, 2)	" 31	Nov. 27	Jan. 13	7½
		1919		
148(1)	Nov. 21	Jan. 4	Feb. 5	8½
Potatoes treated with HCl and dried at 55–60°C.				
		1918	*1918*	
67(1, 2)	Oct. 4	Oct. 5	Nov. 6	4¼
Potatoes treated with acetic acid and dried at 55–60°C.				
52(1, 2)	Sept. 21	Sept. 30	Nov. 2	4¼
Potatoes dried *in vacuo* at 55–60°C.				
			1919	
F	Oct. 15	Dec. 23	Feb. 11	10

sliced uniformly with a slicer in the open air and placed in the drier in very thin layers for desiccation. The baked and dried potatoes were placed in an oven at 204°C., kept there for 45 to 55 minutes until they were soft, then removed and scraped out of the shells, put through a ricer, and placed in the drier in thin layers on cheese-cloth to be dried at 35–40°C.

The Sample F potatoes were desiccated in a vacuum drier after they had been sliced under water and dehydrated at a temperature of 55–60°C. for approximately 10 hours under a pressure of less than 2 inches of mercury.[1]

Two lots of potatoes were treated with acid before drying. One lot was sliced and kept in approximately 2 per cent acetic acid for 18 to 20 hours; the other in approximately 0.2 per cent hydrochloric acid for the same length of time. The slices were then taken out of the acid, rolled in towels, and desiccated in the special drier. Two other lots of potatoes were sliced and steamed for 4 minutes before drying at 55–60°C.

All the dried potatoes were gray-colored except those sliced under water and dried in a vacuum, those treated with acid before drying, those baked and dried, and those steamed and dried. This would seem to indicate that enzyme action had been either inhibited or destroyed in these last named products. Details of drying and using are given in Table I.

RESULTS.

It has been demonstrated that a guinea pig fed the basal soy cake diet *alone* will develop clinical manifestations of scurvy in 2 to 3 weeks. Unless at this juncture proper measures are instituted the animal will die in about a week. A critical review of the appended charts shows the following results with differently treated potatoes tested as antiscorbutic supplements to the basal soy cake diet.

In Chart I it is seen that 10 gm. of raw white potato have protected the guinea pigs against scurvy for the duration of the experiment, 129 days—a period in some cases at least six times as long as that in which scurvy would have developed in the absence of antiscorbutic material.

[1] Our best thanks are due Dr. K. G. Falk for kindly supplying us with this material.

Chart II represents a number of animals which received daily 10 gm. of raw potatoes cooked in water at 100°C. for 15 minutes. There has been no appreciable reduction of the antiscorbutic potency of the potato by this treatment. However, such is not the case if the cooking at 100°C. is continued for 1 hour as shown in Chart IV.

Chart III shows that the antiscorbutic vitamine in raw potatoes cooked for 15 minutes at 100°C. in a weak acid solution, as 0.5 per cent citric acid, is not entirely destroyed by such treatment.

Chart V shows the outcome of feeding daily 2.5 gm. of vacuum-dried potatoes heated in water for 15 minutes at 100°C. The results are not conclusive but indicate that a great part of the antiscorbutic vitamine has been destroyed by this treatment.

Chart VI represents a set of animals to which were fed potatoes dried at 35–40°C. for 6 to 8 hours. Such treatment yields a dried potato which has lost a significant amount of its antiscorbutic potency. A large enough supplement of such potato may afford a slight protection but if the product is heated for 15 minutes in water at 100°C. its value as an antiscorbutic agent is further lessened (Chart VII).

Chart VIII demonstrates that potatoes dried in a blast of air at 55–60°C. for 4 to 6 hours retain some of the antiscorbutic vitamine. However, the quantity of this unknown is further reduced by heating the potatoes in water at 100°C. for 15 minutes (Chart IX).

Charts X and XI show that potatoes dried at 75–80°C. for 2 to 3 hours do not retain so much of the antiscorbutic vitamine as potatoes dried at 55–60°C. for 4 to 6 hours. This statement is based on the fact that several animals showed signs of scurvy on the raw dried potatoes (Chart X) and those guinea pigs represented in Chart XI died in about the usual length of time from the disease. Both of these facts are in striking contrast to the results in Charts VIII and IX.

Chart XII shows that potatoes first heated in the skins at a high temperature and then dried at a low temperature retain enough of the antiscorbutic vitamine in the daily allotment fed to afford the animals protection against scurvy.

Charts XIII and XIV show that treatment with dilute acid (either mineral or organic) before drying does not improve the

method of desiccation so far as the antiscorbutic vitamine is concerned.

Chart XV shows the result of feeding potatoes steamed 4 minutes and then dried at 55–60°C. This procedure apparently does not destroy all the antiscorbutic vitamine as some protection was afforded the animals.

Chart XVI shows that potatoes steamed 4 minutes and then dried at 55–60°C. and cooked 15 minutes in boiling water are no longer antiscorbutic agents.

Chart XVII shows that the skins from baked potatoes when dried at 35–40°C. possess no antiscorbutic value.

DISCUSSION.

Attempts have been made by Holst and Frölich (8) and by Chick and Hume (9) to demonstrate experimentally with guinea pigs that raw potatoes contain antiscorbutic material. These investigators failed to succeed because they could not induce the animals to eat the raw food. A little difficulty of this nature was experienced by us with only one animal (Chart I). The reason for our success is attributed to the fact that animals 6 to 8 weeks of age were used and presumably they had not yet developed a discriminative taste. The amount of raw potato fed per guinea pig per day (10 gm.) was the same as has been reported satisfactory for cabbage and tomatoes. That the actual intake of antiscorbutic vitamine was not the same per gm. of substance of each of these foods is very probable if we associate this substance with either the water or total solid content of the foods. The minimal amount of raw potato which will protect a guinea pig against scurvy has not been determined. However, that slightly less than 10 gm. per day is about the lower limit to suffice for protection is indicated from the feeding curve of Animal 204 which from the 35th day to the termination of the experiment, 108 days, consumed on an average about 7 gm. of raw potato daily.

The influence of heat upon the antiscorbutic vitamine appears to be related not only to the degree of temperature but to the duration of the treatment, the reaction, the enzymes present, and the manner of heating.

A temperature of 35–40°C. for 6 to 8 hours seems to be more destructive than 55–60°C. for 4 to 6 hours, and the latter is scarcely as destructive as 75–80°C. for 2 to 3 hours. This conclusion is based on the results obtained with potatoes dried at these temperatures and fed uncooked and cooked for 15 minutes at 100°C. With 2.5 gm. of potatoes dried at 35–40°C. and fed without further treatment, death from scurvy was slightly delayed; with 5 gm. life was certainly prolonged. One out of four animals on a daily dose of 2.5 gm. of potatoes dried at 55–60°C. showed signs of scurvy at death; also one out of three animals on potatoes dried at 75–80°C. showed signs of scurvy at death. If these dried products are further heated in water at a temperature of 100°C. for 15 minutes and then fed to guinea pigs in amounts equivalent to 2.5 gm. of dried material, there is certainly no protection with the 35–40°C. and 75–80°C. products while there is some with the 55–60°C. material.

When potatoes are baked for a short time at a high temperature and then dried, a product is obtained which affords protection against scurvy. Thus it appears possible that the factors involved in the destruction of the antiscorbutic vitamine are not only the degree of heat and the duration of the heating but also the enzyme content and the reaction of the food being dried. Until further light is thrown upon the problem it seems plausible to assume that at any temperature below 80°C. the enzymes are functionating. For example, all potatoes dehydrated below this temperature darkened during the desiccation. Furthermore, samples of these same products tested 9 months after drying showed that they still contained oxidases. The temperature of 35–40°C. is probably the optimum one for the enzyme action; and the duration of drying at this temperature being very long allows several hours for continuous activity. In the case of drying at 55–60°C. and 75–80°C. there is undoubtedly some enzyme action, but the time is reduced. The slight difference in the antiscorbutic value between the products dried at 55–60°C. and at 75–80°C. and then cooked may be accounted for on the basis that at the higher temperature there is more destruction due to the duration of this greater heating and to the reaction. In the case of baking and drying the enzymes are destroyed in a very short time; also the product is subjected to the high tem-

perature for a short period and thus high heat and reaction have little time to act together.

It was thought that some light could be thrown upon the possible effect of enzyme activity and reaction by steaming the potatoes and by treating them with dilute acids before drying. These procedures undoubtedly destroyed the enzymes and permitted a distinct acid reaction during the course of drying, as proved by proper tests. Nevertheless the resultant dried products were unsatisfactory. The above manipulations are open to criticism in several respects: the acid may have extracted most of the antiscorbutic material; or it may have decomposed it; or the combined heat and acid may have destroyed the vitamine.

Holst and Frölich thought that "the active constituent of antiscorbutic food must be of an enzyme nature because exceedingly small amounts of antiscorbutic food exercise such specific action and the substance in some cases is thermolabile." The results with raw potatoes heated at 100°C. and at 204°C. and dried do not support such a contention; they do, however, lend defense to the idea that enzymes under favorable conditions of temperature and reaction may play a rôle in the destruction of the antiscorbutic vitamine.

What is the relation of growth and maintenance to the experimental diet employed and to scurvy? Givens and Cohen have found that their basal diet was perfectly satisfactory to produce normal growth in the rat. It is then logical to assume that the diet contains among its satisfactory components the fat-soluble and water-soluble vitamines. This idea is further supported by the fact that Givens and McClugage were able to maintain pigeons in good health for a very long period on this diet. If this diet is fed alone to guinea pigs scurvy will develop. Growth, as indicated by an increase in weight, will proceed for a short time during the development of scurvy and is arrested only when the disease has progressed to a marked stage. Maintenance can be secured for only a few days during the rapid development of the malnutrition. A decline sets in very shortly and death soon follows unless a suitable addition of antiscorbutic vitamine to the dietary is made.

The experiments herein reported lend striking confirmation to the belief that there is a third type of vitamine—one protecting

against scurvy. Osborne and Mendel (11) have found from long experience that 0.2 gm. of dried brewer's yeast per rat per day is sufficient to promote normal growth. The basal diet used for producing scurvy contained more than twice that amount. There is no basis to warrant the allegation that the drying of the basal diet or potatoes has destroyed either the fat- or water-soluble vitamine. Chick and Hume (9) and more recently Daniels and McClurg (12) have shown that the water-soluble vitamine is not destroyed by long exposure to a temperature of 100°C. Furthermore Osborne and Mendel (13) have reported that drying spinach at 55–60°C. does not apparently destroy either of the above named factors.

In an interesting study of the dietary properties of the potato McCollum and associates (7) have demonstrated that this food is deficient in calcium, sodium, chlorine, the quality of protein, and the fat-soluble vitamine. All these limiting factors have been supplied in the basal diet used in our experiments.

Holst and Frölich were unable to induce guinea pigs to eat either fresh uncooked or dried uncooked potatoes. They therefore resorted to the use of cooked potatoes. The cooking was for approximately ½ hour in salt water. As dried products they used commercially dried potatoes (concerning which no details are given other than the potatoes were first soaked in dilute sulfuric or hydrochloric acid before dehydrating), potatoes dried in the air at 37°C., and some dried *in vacuo* at 30°C. The animals received no other food except potatoes. With fresh cooked potatoes given *ad libitum* there was apparently almost complete protection from scurvy up to 127 days. However, death ensued sooner or later and there was a marked drop in weight of all animals. This can be explained now in the light of McCollum's experiments with rats whereby it has been determined that the potato is deficient in several respects. Holst and Frölich found with potatoes commercially dried, air-dried at 37°C., and vacuum-dried at 30°C. and then cooked that there was no protection against scurvy.

Our results with fresh potatoes cooked and with potatoes dried at 35–40°C. and cooked confirm those of Holst and Frölich. With fresh potatoes cooked the findings herein reported are in accord with the experience of Chick and Hume.

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Key to Charts.

Sup.	= Supplemental intake.
S. C	= Basal soy cake diet.
Pn.	= Pneumonia.
S	= Scurvy.
S?	= Scurvy questionable.
Pn.S?	= Pneumonia with questionable scurvy.
Pn I.	= Pneumonia and impaction of the intestines.
I	= Impaction of the intestines, cecum.
?	= Cause of death a question.
OK	= Animal apparently recovered.
*	= Termination of the experiment.
†	= Death.

The growth curves, which are self-explanatory, are separated from the feeding curves by a large heavy base line which extends out to the left under the weight figures. The feeding curves, always at the bottom of the chart, are plotted on a basis of 4. In these curves there are five equal subdivisions between the base lines. Consequently if an animal were eating all his food the curves of supplemental and basal intakes would be parallel to the base lines four-fifths of the distance above them. The number of the animal to which the feeding curves belong is always placed on the left opposite the base line separating the curve of supplemental intake (Sup.) from that of the intake of the basal diet (S. C). The curve of the supplemental intake is always above that of the basal food intake. The time relation is the same for all curves; that is, 20 days to each square.

As an example of the food curves, take No. 203, Chart I. On the 1st day this animal ate one-fourth his supplemental intake, on the 2nd and 3rd days one-half, and on the 4th day and every day thereafter he ate all of it. The basal diet curves (S. C) are read in the same way depending upon the depth of the break on the basis of 4.

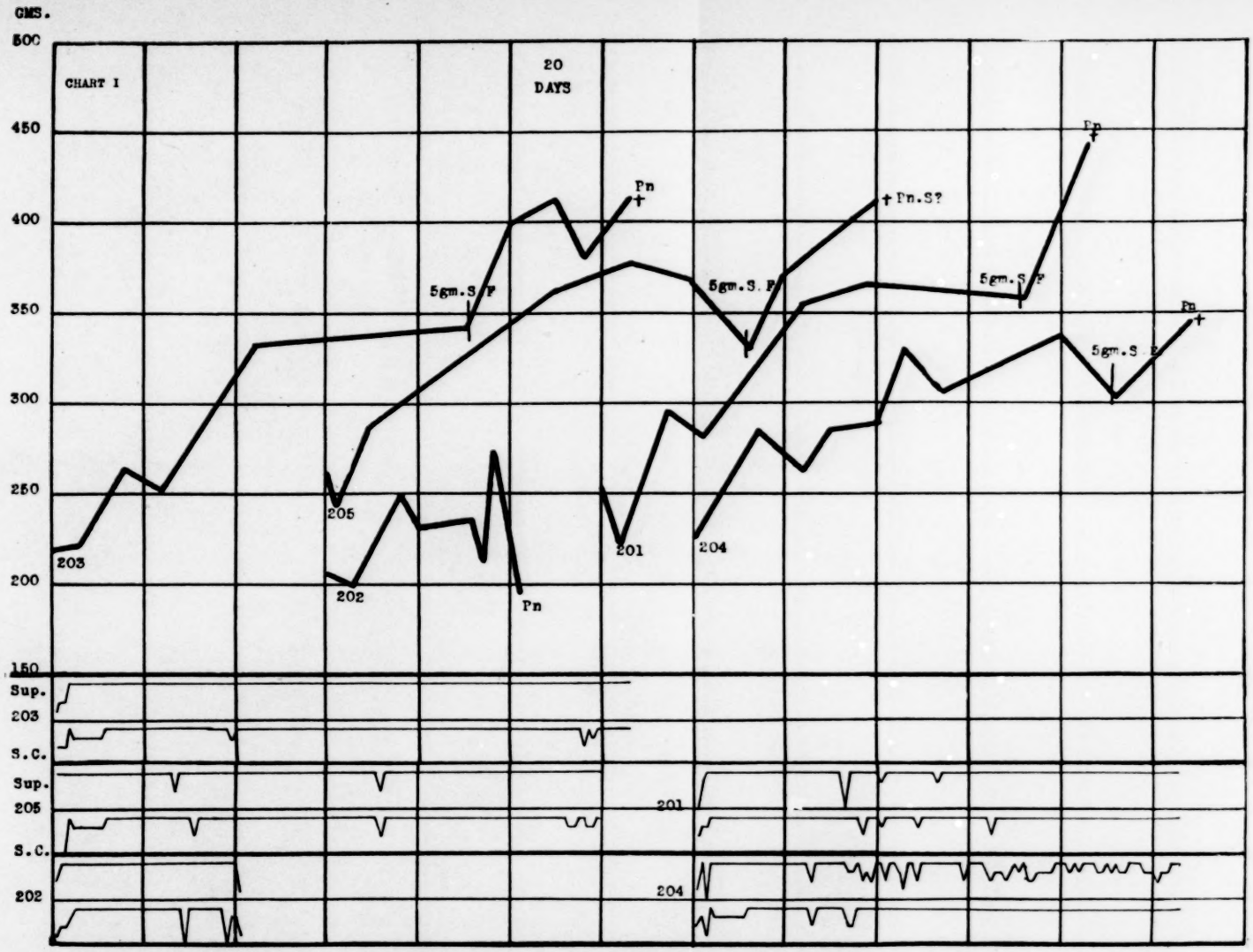


CHART I. This chart shows that a small daily allotment of raw potatoes will prevent the occurrence of scurvy in guinea pigs. 10 gm. of raw potatoes were consumed by each animal except No. 204 whose average intake was about 7 gm. However, this lessened intake did prevent the appearance of any external scorbutic manifestations within the experimental period. Pneumonia accounted for the death of all animals in this group. In the case of No. 205 death was undoubtedly due to pneumonia but whether it was complicated by scurvy is a question. At autopsy this animal showed a few subcutaneous hemorrhages. As the animals increased in weight and age more food was needed as is shown by the way all of them responded to a daily addition of 5 gm. of soy bean flour heated for 30 minutes at 20 pounds pressure.

5 gm. S F = 5 gm. of heated soy bean flour added to the diet.

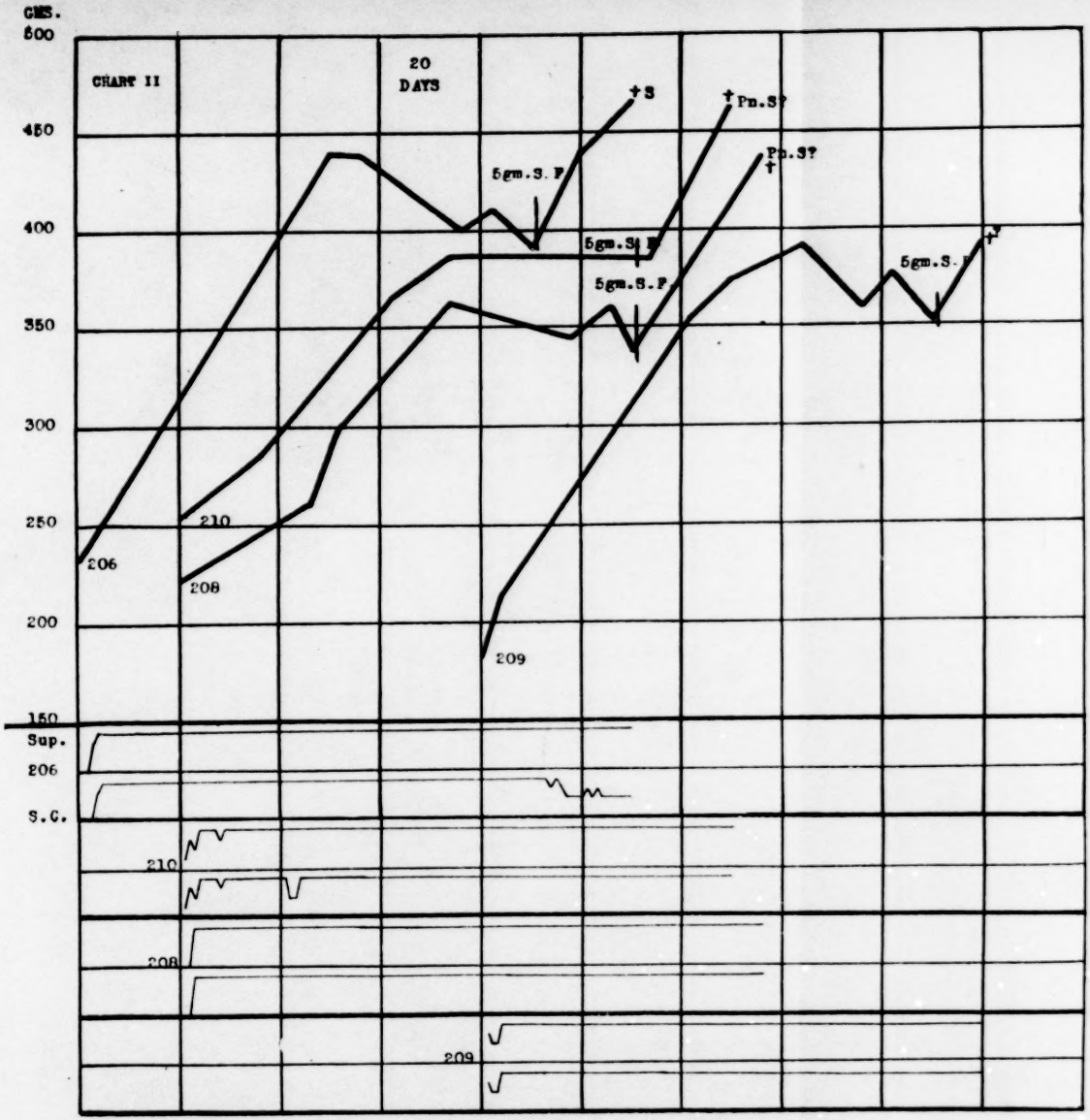


CHART II. These curves show that a daily allotment of 10 gm. of raw potatoes cooked for 15 minutes will protect guinea pigs against scurvy for a very long period. Most of these animals lived as long as those on raw potatoes, in Chart I, but one guinea pig (No. 206) had definite signs and two others questionable evidences of scurvy at death. It therefore seems safe to conclude that even heating the potato at 100°C. for 15 minutes does slightly diminish the content of antiscorbutic vitamine.

5 gm. S F = 5 gm. of heated soy bean flour added to the diet.

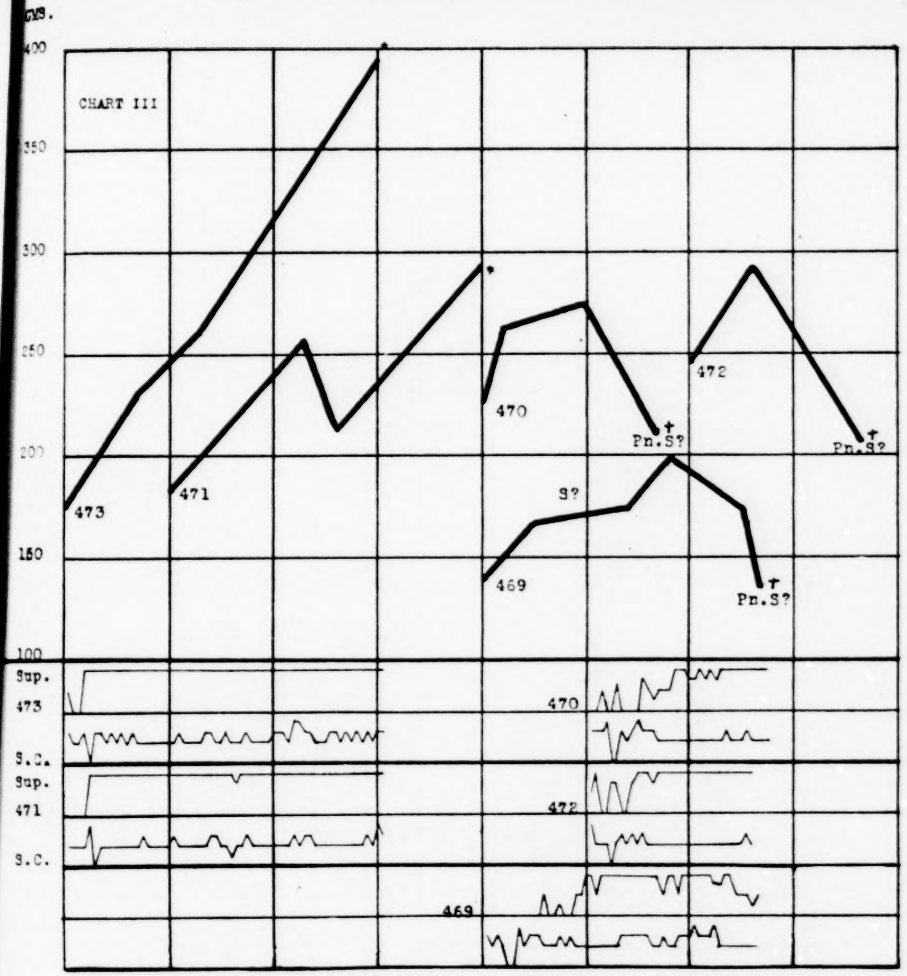


CHART III. 10 gm. of fresh potatoes were cooked for 15 minutes in 11 cc. of 0.5 per cent citric acid and fed to the guinea pigs in this group. The volume of diluted acid chosen was such as to yield a very thick paste at the end of the cooking time. Unfortunately only two of the animals in this group consumed the entire daily supplement of potatoes and the experiments had to be terminated at 60 days for business reasons. However, the indications are that the amount of citric acid used was not destructive and it may have been beneficial. That the latter may be the case when the cooking is for 1 hour is noted in Chart IV.

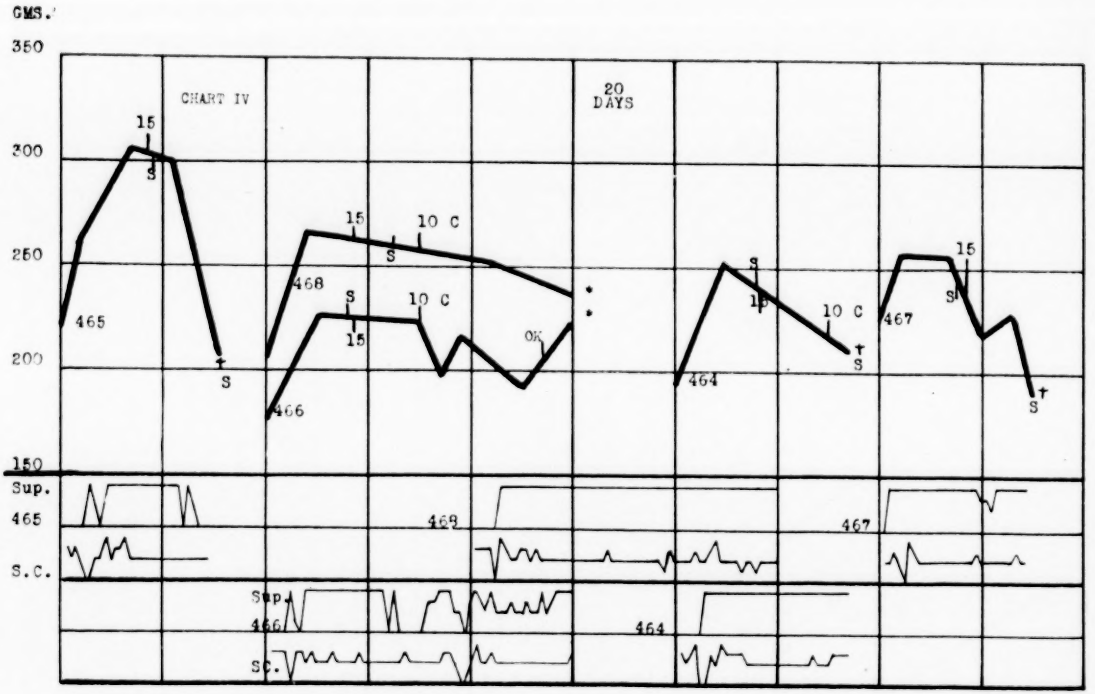


CHART IV. These curves show that 10 gm. of potatoes cooked for 1 hour at 100°C. have their vitamine content so much reduced that scurvy is not prevented. The disease could not be arrested by feeding 15 gm. of potatoes so cooked. In the case of Nos. 466 and 468 scurvy was checked by feeding 10 gm. of potatoes cooked in 11 cc. of 0.5 per cent citric acid for 1 hour. This would seem to indicate that a slight organic acidity may prevent some of the deleterious action of heat upon the antiscorbutic vitamine.

15 = 15 gm. of potatoes.

10 C = 10 gm. of potatoes cooked in 11 cc. of 0.5 per cent citric acid 1 hour.

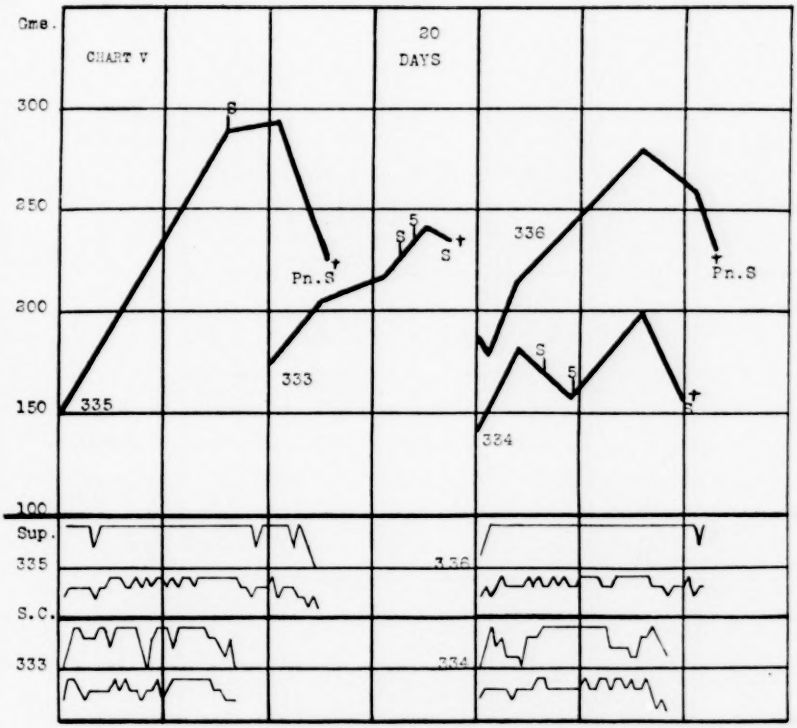


CHART V. These curves represent the result of feeding potatoes dehydrated at a low temperature *in vacuo*. This mode of desiccating potatoes yields a product which is not entirely devoid of some antiscorbutic property if one can judge by the fact that the onset of scurvy has been delayed.

5 = 5 gm. or a double dose of potatoes fed.

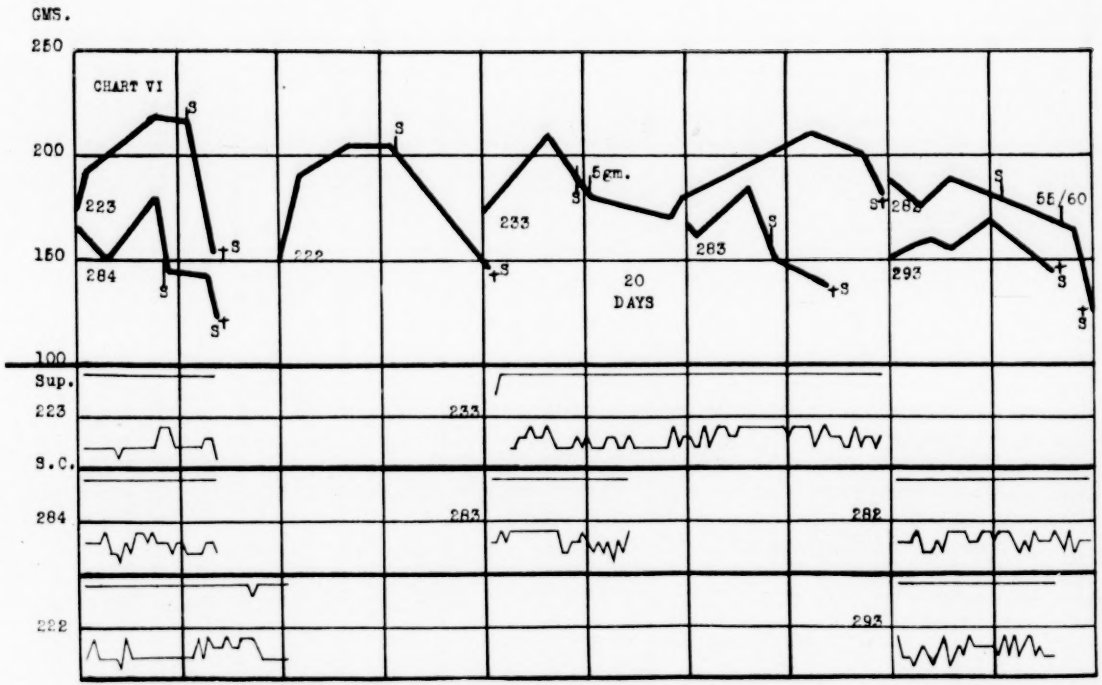


CHART VI. The animals in this group were fed potatoes which had been dehydrated at 35–40°C. Such a product contains practically no antiscorbutic potency. That it is not entirely free of the antiscorbutic vitamine is shown in the case of No. 233 which was fed 5 gm. of the dried potatoes daily after the appearance of scurvy. This animal was maintained for 60 days after the appearance of the disease but finally succumbed regardless of the double daily dose of dried potatoes.

5 gm. = 5 gm. or a double dose of potatoes fed.

55/60 = Potatoes dried at 55–60°C. substituted for those dried at 35–40°C.

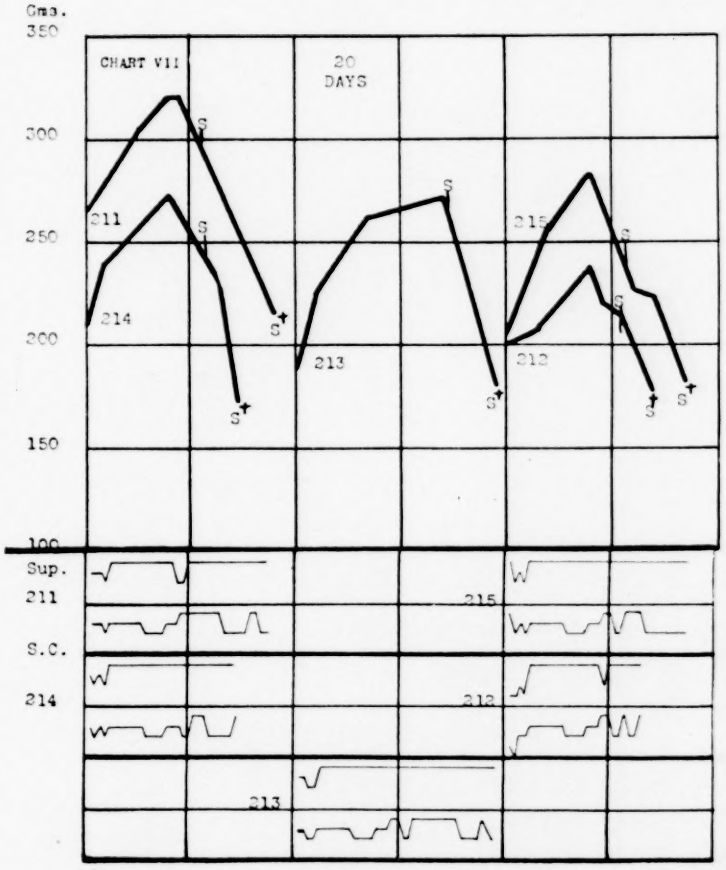
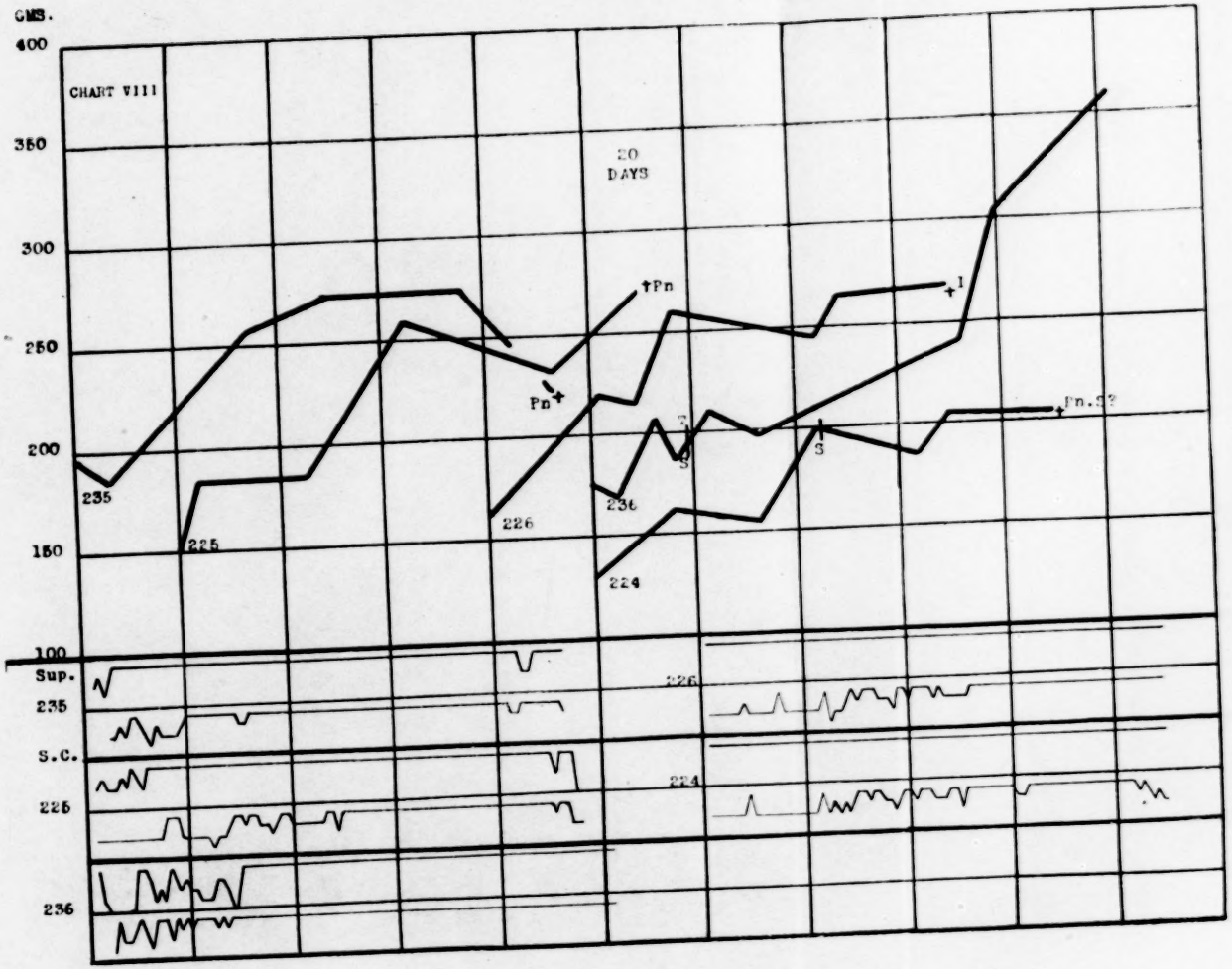


CHART VII. These curves show that potatoes dehydrated at 35–40°C. and then cooked for 15 minutes at 100°C. afford no protection against scurvy. The amount here fed corresponds to 10 gm. of raw potatoes which, as is seen from Chart I, will protect guinea pigs against the disease.

55 60 = Potatoes dried at 55–60°C. substituted for those dried at 35–40°C.

CHART VIII. These curves demonstrate that potatoes dehydrated at 55–60°C. contain enough of the antiscorbutic essential to prevent the development of scurvy over a period three times as long as that in which the disease would appear if no protection were afforded. Three of the animals (Nos. 235, 225, 226) showed no signs of scurvy at the time of death (90 days after beginning the experiment); succumbing in the case of the first two to pneumonia and the other to an apparent impaction. No. 236 would not eat the potatoes and accordingly developed scurvy; with 10 gm. of fresh tomatoes cooked 15 minutes at 100°C. it was cured and carried for over 100 days when removed from the experiment. No. 224 showed some external indications of scurvy, as tender joints, at 45 days; however, it shortly improved and at death, 45 days later, showed questionable signs of scurvy.

T = Tomatoes introduced into the diet.


GMS.
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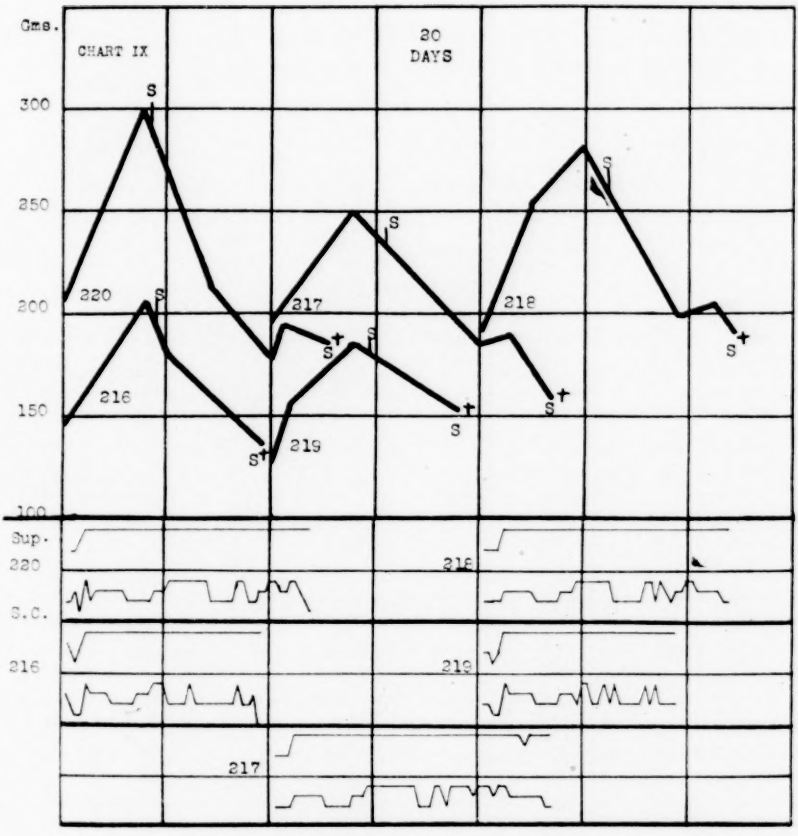


CHART IX. Potatoes dehydrated at 55–60°C. and then cooked for 15 minutes at 100°C. were fed the guinea pigs represented by the curves. The daily supplement was 2.5 gm. of dried material. None of the animals was protected against scurvy. Such antiscorbutic potency as may remain after dehydration at 55–60°C. is apparently rendered *nil* by the short cooking process at 100°C.

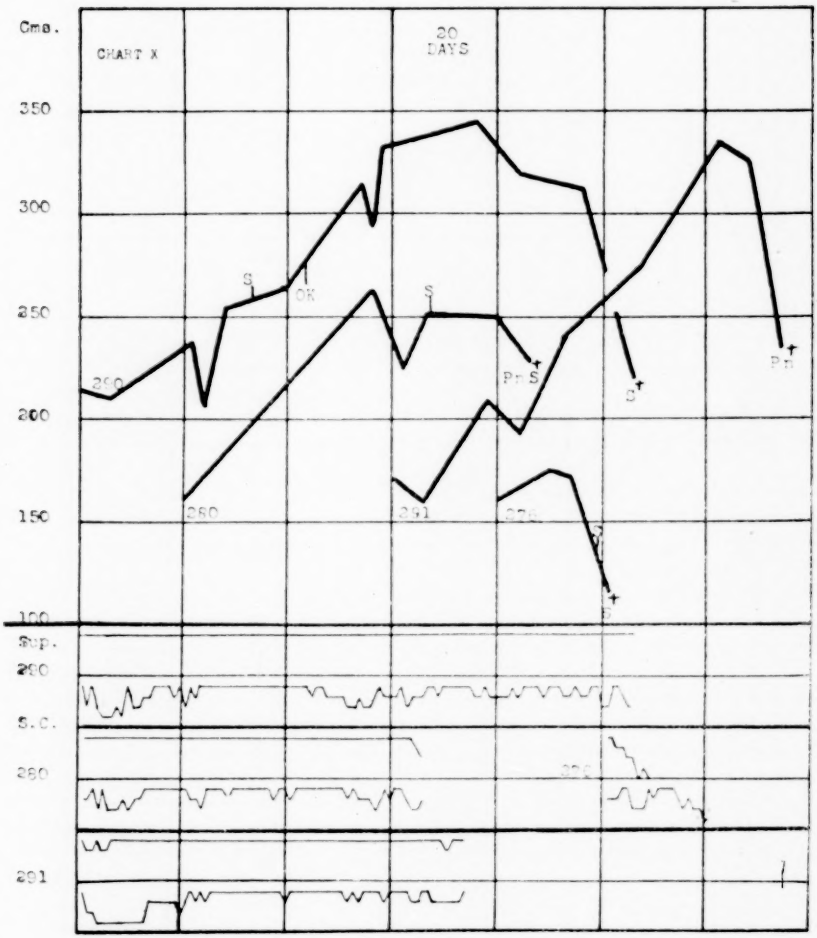


CHART X. 2.5 gm. of potatoes dehydrated at 75–80°C. were fed as a daily allotment to this group of animals. Such a product undoubtedly contains some of the antiscorbutic vitamine but the amount is certainly not equivalent to that contained in potatoes dried at 55–60°C.

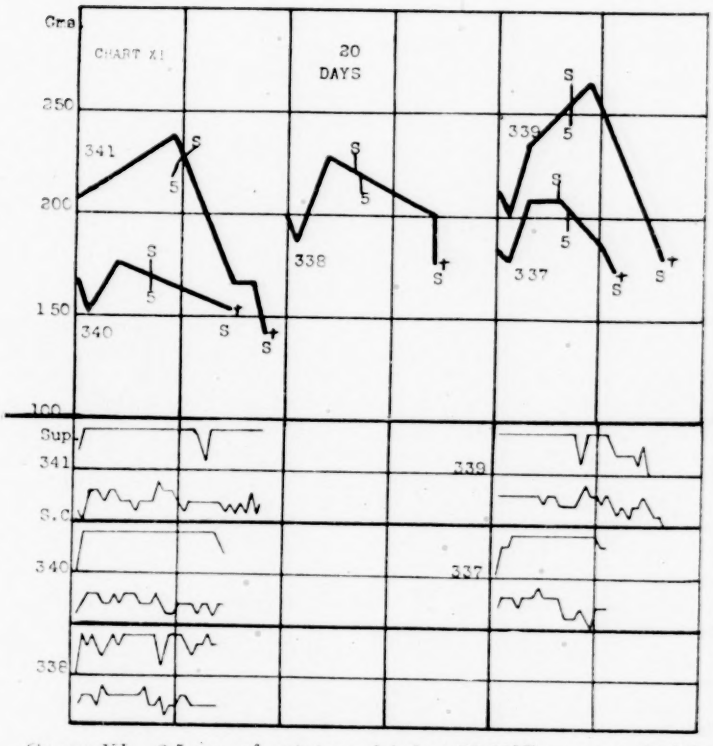
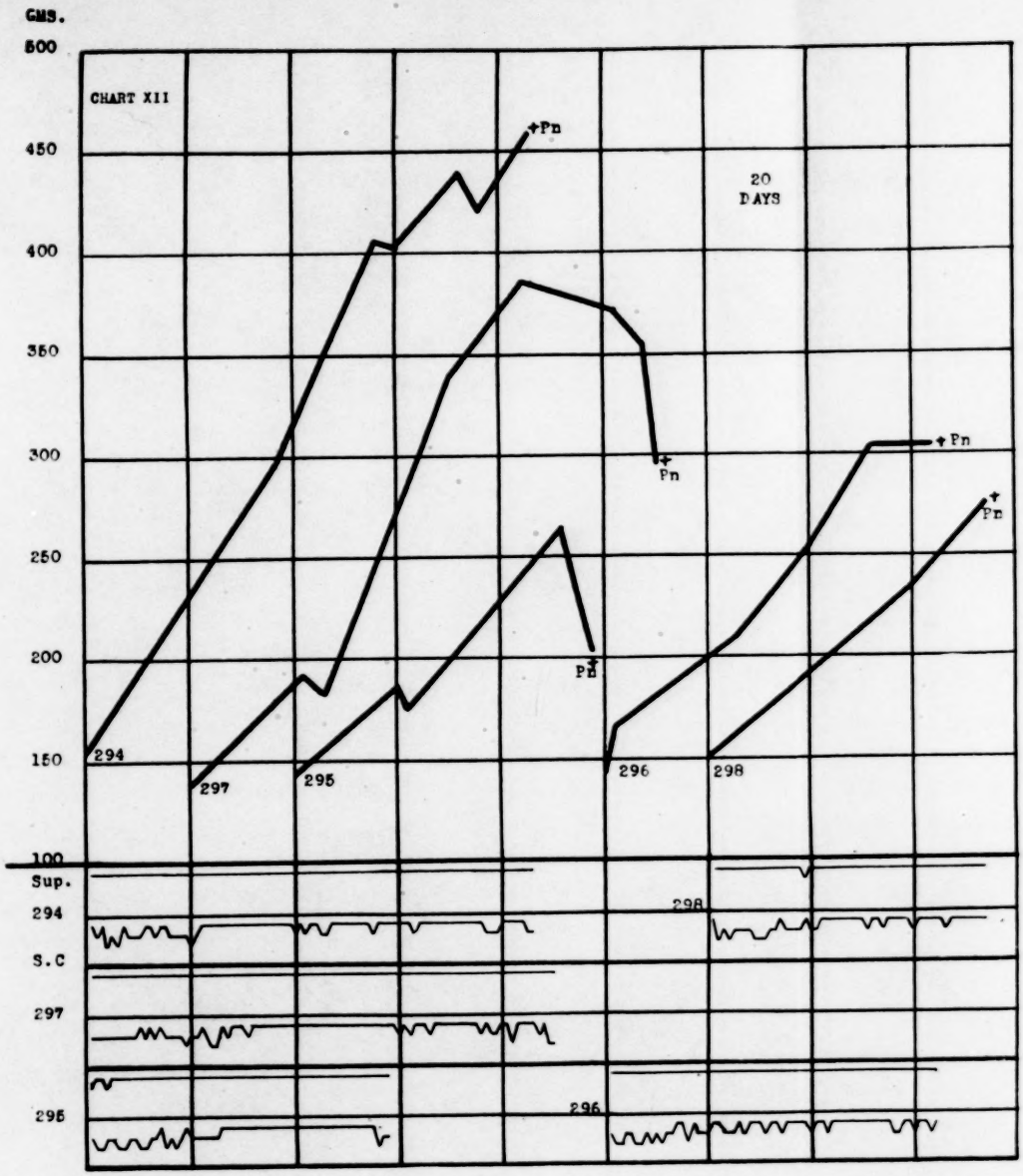


CHART XI. 2.5 gm. of potatoes dried at 75–80°C. were cooked for 15 minutes at 100°C. and fed to this group of guinea pigs. No protection against scurvy was afforded the animals even when the dosage was doubled. Again the destructive effect of cooking at 100°C. on the antiscorbutic vitamine is shown.

5 = 5 gm. or a double dose of potatoes fed.

CHART XII. Potatoes were baked in the skins for 45 to 55 minutes at 204°C., then scooped out, and dried at 35–40°C. This dried product was fed in 2.5 gm. doses daily and protected the guinea pigs against scurvy. All the animals died from pneumonia showing none of the lesions of scurvy. It seems indicated from these experiments that subjection for a short time to a high temperature and then drying at a low one is less destructive than drying at a low temperature alone.


GMS.
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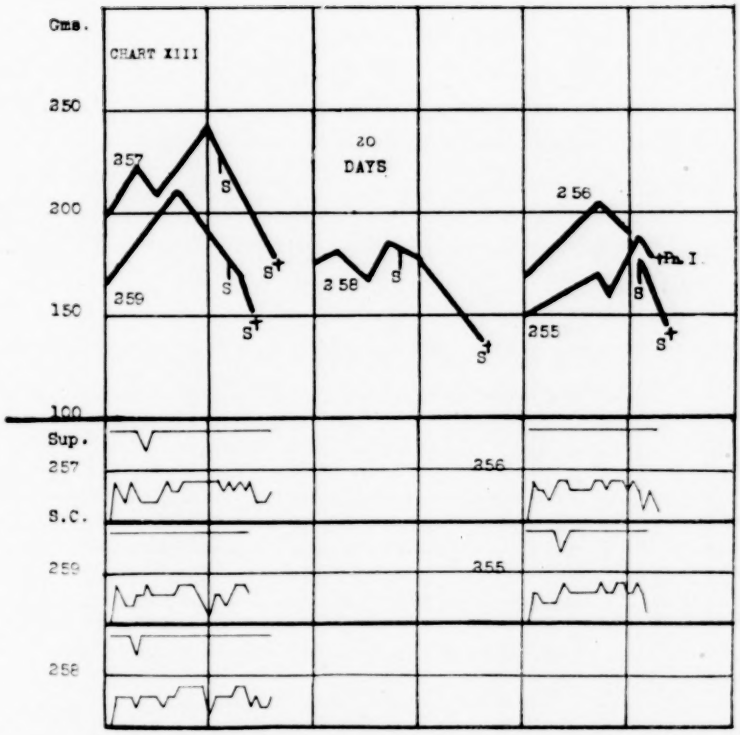

CHART XIII. Potatoes were soaked over night in dilute hydrochloric acid, dried at 55-60°C., and then cooked for 15 minutes at 100°C 2.5 gm. of dried material so treated afforded no protection against scurvy. In previous experiments (Chart IX) it is seen that the animals lived longer on the potatoes dried at 55-60°C. and cooked 15 minutes than the animals represented by the above curves. The mineral acid in connection with the heat employed or the extraction of the vitamine by the acid treatment or both factors combined lessened the antiscorbutic potency of the product.

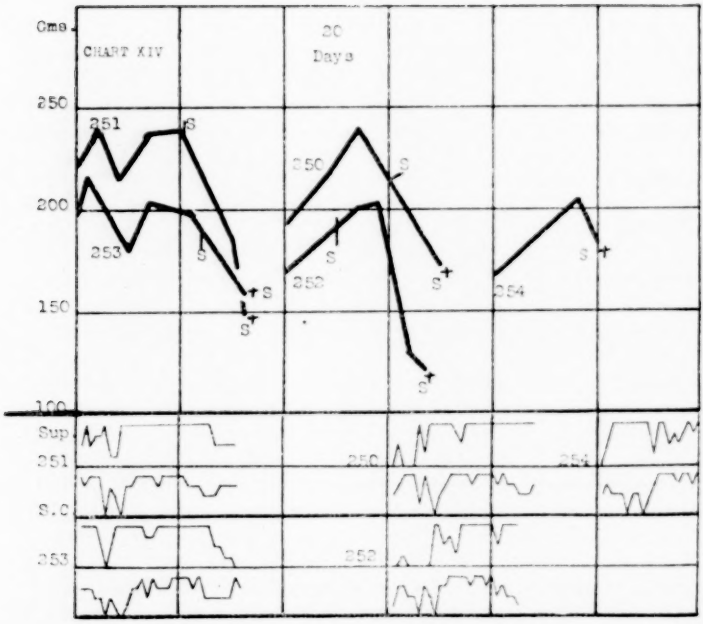

CHART XIV. Potatoes soaked over night in dilute acetic acid, dried at 55–60°C., and then cooked for 15 minutes at 100°C. were fed to guinea pigs in this group. This product was ineffective in preventing scurvy.

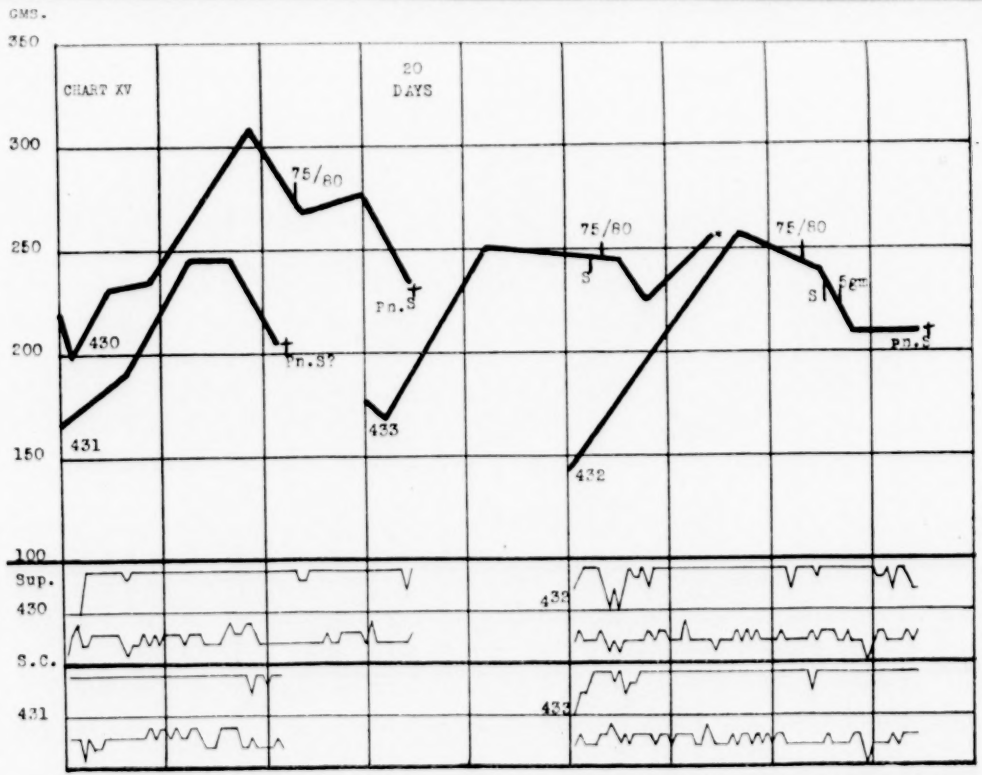

CHART XV. Potatoes steamed for 4 minutes and then dried at 55–60°C. were fed to the guinea pigs represented by the above curves. One animal died at 43 days from pneumonia showing signs of scurvy. Accordingly all animals were transferred to 2.5 gm. of potatoes dried at 75–80°C. Two of them died from pneumonia showing scorbutic symptoms; the other one was apparently improved at 70 days when the experiment had to be terminated.

75 80 = Potatoes dried at 75–80°C. substituted for those dried at 55–60°C. after a previous steaming for 4 minutes.

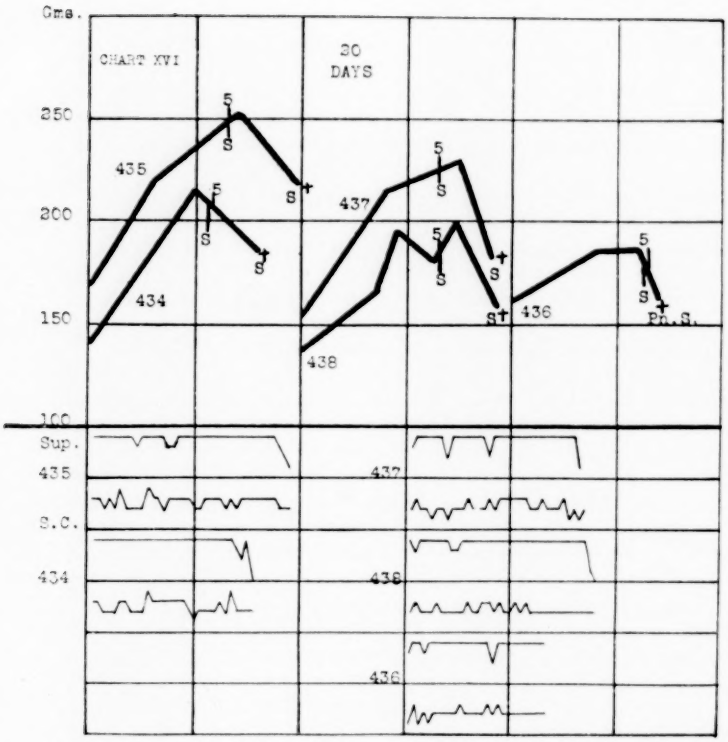

CHART XVI. Potatoes were steamed for 4 minutes, dried at 55–60°C., and cooked for 15 minutes at 100°C. A daily dose equivalent to 2.5 gm. of dried material failed to afford these animals any protection against scurvy, even when the dose was doubled.

5 = 5 gm. or a double dose of potatoes fed.

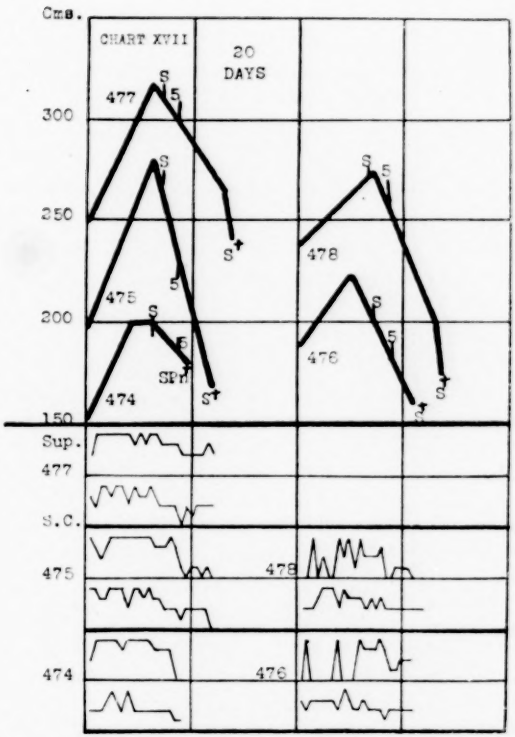

CHART XVII. The potato shells from the baked potatoes were dried at 35–40°C. and 2.5 gm. fed daily. This material even in a double dose failed to protect the animals against scurvy.

5 = 5 gm. or a double dose of potato shells fed.

NEUTRALITY REGULATION IN CATTLE.*

By N. R. BLATHERWICK.

(From the Research Laboratories of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture.)

(Received for publication, May 6, 1920.)

Subsequent to the publication of a method for the determination of the carbon dioxide-combining power of blood plasma by Van Slyke (1917), much of value has been contributed to our knowledge of the alkaline reserve of the animal body. Normal values and the changes produced by various pathological and experimental conditions have been established for a number of mammals. Naturally, the changes occurring in man have been most studied, and the amount of carbon dioxide which the plasma is able to hold in combination as bicarbonate is justly regarded as a convenient and accurate measurement of the alkaline reserve of the body.

As far as I have learned, no one has attempted a study of the alkaline reserve of cattle. In the course of an investigation of the physiology of milk secretion in progress in this laboratory, it became of great importance to discover the changes occurring in the acid-base balance of cattle, and to attempt to gain an insight into the manner in which these animals maintain their tissue neutrality.

It has long been a well known fact that cows normally excrete a strongly alkaline urine which is loaded with carbonate, as shown by the violent ebullition of carbon dioxide when an acid is added. This strongly alkaline reaction is justly referred to the diet of cows, one in which there is commonly a large excess of basic elements. On the other hand, calves which consume an approximately neutral diet, milk, commonly excrete urines that are neutral or amphoteric to litmus. Judging from these facts one would expect calves to have a smaller alkaline reserve than cows. In-

* Published with the permission of the Secretary of Agriculture.

deed there are many observations on man that lend support to this hypothesis. Marriott (1916, *a*) has shown that the blood of infants has a higher "fixed acidity" than that of adults, and that the alveolar CO_2 tension of infants is low (1916, *b*). Children are known to be especially susceptible to acidotic conditions.

Meigs, Blatherwick, and Cary (1919, *a*) have pointed out that the plasma of young calves contains more calcium than that of cows, and have suggested that this might be connected with a lower CO_2 capacity in the case of calves. Rona and Takahashi (1913) studied the solubility of calcium in solutions of $NaHCO_3$ and showed that if the concentration of hydrogen ions remains constant the amount of calcium in solution varies inversely with the bicarbonate. This taken with the observations of Allers and Bondi (1907), who were able to double the concentration of the blood calcium by feeding hydrochloric acid to rabbits, made it desirable to determine the concentration of calcium and the carbon dioxide-combining power of plasma under various conditions. In several instances, estimations of the phosphorus content of plasma, and of the urinary excretion of calcium, phosphorus, ammonia, and carbon dioxide have been made.

Methods.

Carbon dioxide-combining power of plasma was determined by the well known method of Van Slyke (1917), calcium and phosphorus by the methods described by Meigs, Blatherwick, and Cary (1919, *a*, *b*), and ammonia in the urine by Steel's (1910) modification of the Folin procedure. Estimations of the carbon dioxide content of urine were made in the Van Slyke apparatus. Many urines contain so much carbonate that a 1:4 dilution must be made and the determination carried out on this solution.

Blood samples were obtained at about 9 a.m., approximately 4 to 5 hours after the last feeding. Blood was taken from the jugular vein through a large cannula. When blood is allowed to flow rapidly through a large cannula in this manner and is immediately centrifuged there is no appreciable loss of carbon dioxide. I have several times made check determinations with samples taken under paraffin oil and am fully convinced of this fact. Plasma from blood to which saturated sodium citrate so-

lution is added in 1 per cent concentration to prevent coagulation also gives the same values for CO_2 capacity, as does the corresponding plasma in which sodium oxalate is used as an anticoagulant.

On account of the inherent difficulties of obtaining 24 hour specimens of urine from cows, I have collected the first urine voided after the blood sample was secured. It is realized that the values from single specimens may vary somewhat from those obtaining for the urine of a complete day. However, it is thought that such results represent actual conditions closely enough for the purposes in view.

Carbon Dioxide Capacity of Cow Plasma.

Examination of the values shown in Table I reveals the remarkable constancy of the alkaline reserve of the cow. Twenty-two determinations of the CO_2 capacity of sixteen individuals gave an average value of 61.5 cc. of CO_2 bound by 100 cc. of plasma. The maximum and minimum figures were 68.3 and 55.1 cc., respectively. The table is arranged beginning with the lowest CO_2 capacity and extending to the highest, except when more than one determination has been made at a different date on an animal, in which case the values follow chronologically. No evidence of the acidosis of pregnancy observed in women by Hasselbalch and Gammeltoft (1915), and by Losee and Van Slyke (1917) is indicated by the CO_2-combining power of the plasma of pregnant cows. In fact, the majority of pregnant animals gave values about or higher than the average; *i.e.*, a greater alkaline reserve. None of the figures here given can be interpreted as showing the presence of an acidotic condition, and it may be justly said that the determination of plasma CO_2 capacity of cows gives no indication of slight tendencies in this direction. The variations in the alkaline reserve are such as can be duplicated by appropriate changes in rations, as will be demonstrated in another part of this paper.

TABLE I.

Carbon Dioxide-Combining Power of Cow Plasma.

Date.	Cow No.	CO_2 capacity per 100 cc.	Remarks.
		cc.	
Aug. 11, 1919	121	58.9	Farrow.
Oct. 9, 1919	121	55.1	"
June 26, 1919	119	56.0	No morning feed. Pregnant, 7th month.
Sept. 8, 1919	84	56.9	No morning feed. Farrow.
Nov. 13, 1919	84	57.9	CO_2 in urine, 247 cc. per 100 cc. Farrow.
Jan. 14, 1920	84	63.6	Farrow.
Sept. 15, 1919	89	58.9	Pregnant, 3rd month.
Jan. 7, 1920	89	64.0	" 7th "
Feb. 17, 1920	63	59.5	Not lactating.
Sept. 10, 1919	88	59.7	Pregnant.
Nov. 18, 1919	88	62.6	CO_2 in urine, 235 cc. per 100 cc. Pregnant.
Feb. 25, 1920	88	61.7	Pregnant. Due to calve March 19.
" 13, 1920	221	60.3	Pregnant, 8th month.
Aug. 22, 1919	114	60.6	Aborted 2 days before; 5th month of pregnancy.
" 19, 1919	220	62.6	Farrow.
" 27, 1919	249	63.1	Calved 10 days later.
Oct. 15, 1919	64	64.5	Milking.
June 19, 1919	82	64.5	Farrow.
Aug. 26, 1919	244	64.5	Calved 1 day later.
Dec. 3, 1919	56	64.6	Farrow.
Nov. 28, 1919	51	65.5	Milking.
Jan. 15, 1920	49	68.3	Aborted preceding day; about 5 weeks before time.

The Alkaline Reserve of Calves.

Table II gives among other things the amount of CO_2 chemically bound by the plasma of young calves. At a glance one sees that calves possess a larger alkaline reserve than do grown cattle. The average CO_2 capacity of the plasma of seven calves ranging in age from 2 to 14 days is 73.0 cc., with maximum and minimum values of 80.6 and 68.3 cc., respectively. Calf 309, when it was slightly over 1 month old, showed a CO_2 capacity of 61.4 cc., practically identical with the average adult figure.

Attention is also invited to the values obtained for urinary carbon dioxide. Calves excrete approximately neutral urines containing from 3 to 17 cc. of CO_2 per 100 cc., while the strongly alkaline urine of cows usually contains from 200 to 400 cc. of CO_2 in 100 cc. But in spite of this calves have a decidedly greater CO_2 capacity, amounting on the average to a difference of 11.5 cc. per 100 cc. of plasma. It is evident that a fundamentally different regulatory mechanism is functioning in the two cases.

TABLE II.

Composition of Plasma and Urine of Young Calves.

Date.	Calf No.	Age.	Plasma.			Urine.			Remarks.
			CO_2 capacity per 100 cc.	Ca per 100 cc.	P (inorganic) per 100 cc.	Specific gravity.	CO_2 per 100 cc.	NH_3 N per 100 cc.	
1919		*days*	*cc.*	*mg.*	*mg.*		*cc.*	*mg.*	
Sept. 27	309	33	61.4	11.1	7.5	1.006	5.7	17.0	
Aug. 29	309	2	68.3	12.1					Blood obtained after some struggling.
1920									
Feb. 5	314	4	70.2	10.6	6.8	1.015	17.3	9.7	
" 16	310	36	71.0	10.5	7.7	1.007	9.7	14.6	P in urine, 47.3 mg. per 100 cc.
Jan. 13	310	2	74.8	13.2	6.8	1.006	14.0	8.3	
Feb. 9	312	10	71.1	12.4	8.2	1.016	8.8		
" 10	311	14	73.0	11.5	9.3	1.021	5.0		
" 12	315	4	73.0	12.2	6.7	1.006	2.9		
" 7	313	7	80.6	12.3	7.3	1.004	13.3		P in urine, 30.8 mg. per 100 cc.

The values in Table II corroborate and extend the observations of Meigs, Blatherwick, and Cary (1919, *a*), who showed that the plasma of calves is characterized by a high content of calcium and inorganic phosphorus. As was pointed out in that communication, calcium soon begins to decrease toward the adult level but the phosphate gains in amount until the highest point is reached at an age of about 6 months.

The urine of young animals as compared with that of cows contains less carbon dioxide, more ammonia, and more phosphorus. Many cows excrete urines which carry only small amounts of the latter element; about 2 mg. per 100 cc. The urine of calves normally contains from fifteen to twenty times this amount.

The Effect of Individual Foods upon Blood and Urine of Cows.

It was my privilege while working under the direction of Dr. Lafayette B. Mendel to demonstrate the specific effect of individual foods upon the composition of the urine of men (1914). This study revealed the ease with which urinary acidity could be varied by changes in diet. Sherman and Gettler (1912) had previously made more reliable analyses of the ash of most of the common articles of food than had hitherto been available. Their analyses showed a preponderance of basic elements in the ash of vegetables and fruits, and an excess of acid-forming elements in that of meats and cereals. The result of eating the latter is an increased output of acid in the urine, but fruits and vegetables cause the production of less acid or alkaline urines. It was found that certain fruits, such as cranberries, plums, and prunes, although they yield an alkaline ash, nevertheless in metabolism led to an increase in urinary acidity. This anomalous result is doubtless, in part, due to the benzoic acid radical contained in these fruits.

Whether the alkaline reserve of the body can be altered by varying the intake of foods is an open question. Van Slyke and Cullen (1917) state that unusually high values for plasma CO_2 capacity are sometimes observed after the subject has partaken of an alkaline diet. Schloss and Harrington (1919) were able to increase the plasma CO_2-combining power of infants by appropriate changes in diet. On the other hand, McClendon, von Meysenbug, Engstrand, and King (1919) were unable to produce any change in the alkaline reserve of man by varying the diet.

In Tables III and IV are presented the analytical results of experiments which were designed to throw light on this question as well as on several others.

The first experiment on Cow 56 reveals very clearly the changes in the composition of blood and urine that may be produced by

alterations in diet. It may, incidentally, be remarked that this cow was previously fasted for a period of 7 days. Her recovery from the fast was rapid and she was in excellent physical condition at the beginning of and during the present studies. This animal was first placed upon a diet of grain only.

Reference to Table III shows that on March 1, the 4th day on this food, her plasma CO_2 capacity was 58.6 cc. This value falls near the lower limit of those obtained for cows on mixed rations.

TABLE III.

Changes in Blood and Urine Produced by Individual Foods. Cow 56.

Date.	Plasma.					Urine.				Remarks.
	CO_2 capacity per 100 cc.	Ca per 100 cc.	Total P per 100 cc.	Inorganic P per 100 cc.	Lipoid P per 100 cc.	Specific gravity.	CO_2 per 100 cc.	NH_3 N per 100 cc.	P per 100 cc.	
1920	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*		*cc.*	*mg.*	*mg.*	
Mar. 1	58.6	10.3	12.2	5.7	7.5	1.027	37	11	178	Grain only.*
" 4	69.2	11.3	9.6	3.9	5.7	1.030	554	4	3	Alfalfa hay only.†
" 9	54.8	10.4	9.1	3.6	5.5	1.021	4	13	54	Corn silage only.‡

* Amounts of grain eaten:

	lbs.	
Feb. 27	10	(No. 17)
" 28	18	(No. 17)
" 29	15½	(No. 17)
Mar. 1	12	(No. 18)

Composition of mixture No. 17:

Ground oats	100 parts
Bran	100 "
Hominy	100 "
Linseed meal	50 "

† Amounts of alfalfa hay eaten:

	lbs.
Mar. 1	5
" 2	20
" 3 and 4	20½

Composition of mixture No. 18:

Ground oats	100 parts
Corn-meal	100 "
Bran	100 "
Linseed meal	50 "
Cotton-seed meal	50 "

‡ Amounts of corn silage eaten:

	lbs.
Mar. 5	20
" 6	16
" 7	30
" 8	46
" 9	26

Immediately following the acid-forming grain diet, the cow was given alfalfa hay only. Blood taken on March 4 shows that the CO_2 chemically bound by 100 cc. of plasma had increased to a value of 69.2 cc., a change of 10.6 cc. Another sample, obtained on March 9, shows that when corn silage had been the sole food plasma CO_2 capacity decreased to a value of 54.8 cc., lower than that produced by the eating of grain. This last result seems, at first sight, rather surprising but upon study a satisfactory explanation can, I think, be given. Discussion of this point will be deferred to a later part of this communication.

The response of the plasma phosphorus compounds is significant. As a result of the high phosphorus, grain diet, high values for inorganic and lipoid phosphorus were produced. This is a corroboration of the phenomenon previously described by Meigs, Blatherwick, and Cary (1919, *a*). With a change to the low phosphorus food, hay, the inorganic phosphorus is seen to have dropped from 5.7 to 3.9 mg. in 100 cc. of plasma. Still further reductions in plasma phosphorus compounds resulted when corn silage was the sole food. Comparable changes in plasma phosphate may be observed in the case of Cow 66, Table IV.

The response of plasma calcium to changes in food is also noteworthy. When alfalfa hay with its high content of this element was the food, calcium amounted to 11.3 mg. per 100 cc. of plasma, as compared with 10.3 and 10.4 mg. on grain and silage. I have observed another case in which the concentration of calcium was high in an animal fed solely upon hay. In this instance an effort was being made to dry up hastily a cow which was nearly at the end of the gestation period. On the day prior to and on the day of taking the blood sample, this cow ate neither grain nor silage but consumed 16 pounds of alfalfa hay daily. Her plasma contained 12.1 mg. of calcium in 100 cc., as contrasted with the normal value of about 10 mg. These results are in harmony with those of Boggs (1908) who observed an increase of 36 per cent in the calcium content of dog blood as a result of feeding calcium lactate. It would appear that the level of plasma calcium may be influenced by the calcium content of the diet, but in cows on rations quite similar in this respect the concentration is subject to only small variations.

The changes produced by diet in certain constituents of the urine are most interesting. Urine resulting from the grain diet showed an excretion of 37 cc. of CO_2 in 100 cc. With a change to the base-forming food, hay, fifteen times this amount was found (554 cc.). Coincident with an acid urine excreted when the animal ate silage, only 4 cc. of CO_2 were found, less than in

TABLE IV.

Changes in Blood and Urine Produced by Individual Foods. Cow 66.

Date.	Plasma.				Urine.					Remarks.
	CO_2 capacity per 100 cc.	Total P per 100 cc.	Inorganic P per 100 cc.	Lipoid P per 100 cc.	Specific gravity.	CO_2 per 100 cc.	NH_3 N per 100 cc.	Ca per 100 cc.	P per 100 cc.	
1919	*cc.*	*mg.*	*mg.*	*mg.*		*cc.*	*mg.*	*mg.*	*mg.*	
Oct. 16	60.7	10.3	4.9	5.4	1.035	32	25		2.7	Silage 16 lbs., grain 2 lbs.
" 20	64.1	10.0	5.0	5.0		306	6		1.7	Alfalfa hay only.*
" 31	64.5	9.9	4.6	5.3	1.037	358	6	4.8	2.4	Silage 16 lbs., grain 2 lbs.
Nov. 4	63.6	11.1	6.1	5.0	1.035	142	12	3.2	20.2	Grain only.†
" 17	67.3	8.7	3.9	4.8	1.037	385	5	12.0	2.2	Silage 16 lbs., grain 2 lbs.
" 20	61.7	8.2	2.9	5.3	1.036	200	9	50.5	2.5	Silage only.‡

* Amounts of alfalfa hay eaten:

	lbs.
Oct. 16	12
" 17	$8\frac{3}{4}$
" 18	$11\frac{1}{2}$
" 19	8
" 20	$6\frac{1}{4}$

† Amounts of grain eaten:

	lbs.
Nov. 1	10
" 2	14
" 3	18
" 4	6

Composition of grain mixture:

Ground oats	100 parts
Hominy	100 "
Bran	100 "
Linseed meal	50 "

‡ Amounts of silage eaten:

	lbs.
Nov. 17	12
" 18	30
" 19	58
" 20	$4\frac{1}{2}$

the case of grain. Appropriate and corresponding values for ammonia were observed; *i.e.*, 11, 4, and 13 mg. of N in 100 cc. of urine. The urinary excretion of phosphorus reveals some noteworthy features. Upon the high phosphorus, acid-forming, grain diet this cow excreted 178 mg. of P in 100 cc. of urine. This value became 3 mg. in the urine formed from the low phosphorus, base-forming hay. Although the urine from the silage period was more acid than that from the grain, still less than one-third as much phosphorus (54 mg.) was eliminated in this excretion. These figures show conclusively that phosphorus contained in the grain was much more efficiently and rapidly absorbed than it was from either of the other foods.

These and other experiments demonstrate that the amount of phosphorus excreted in the urine is dependent upon the quantity of phosphate in the plasma. But this relation is disturbed when an increased acid production occurs. This is well illustrated by comparing the values obtained from the hay and silage periods. Although the inorganic phosphorus of the plasma was lower when silage was fed, nevertheless, eighteen times as much phosphorus was excreted in this urine owing to the necessity for the elimination of acid. Another factor which doubtless contributed to the low value of 3 mg. in the urine of the hay period was the presence of large amounts of calcium in the food.

The other experiment on Cow 66 was arranged differently. She was placed upon a basal ration of 16 pounds of silage and 2 pounds of grain daily, and after she ate this ration for 4 to 5 days blood and urine samples were obtained. The animal then received only one food, for instance hay, during a period of 3 to 4 days when samples of blood and urine were again taken. Between the experimental periods, the cow was given the basal ration of 16 pounds of silage and 2 pounds of grain. It developed that this basal ration was insufficient for maintenance and the experimental findings must be interpreted with this reservation in mind.

If we turn our attention to the results shown in Table IV, we will observe that the values obtained for the three sets of samples resulting from the basal ration are not comparable. The first samples obtained on October 16 show evidences of acidosis; *i.e.*, low urinary CO_2 and a considerable amount of ammonia. The second and last specimens of urine corresponding to the basal

ration are quite similar to each other as is seen by comparing the values for urinary CO_2 and ammonia. These latter values are evidently the correct ones for this basal diet. For some unknown reason, the animal was mildly acidotic when the first samples were obtained.

In spite of the fact that this cow ate but sparingly of alfalfa hay, $11\frac{1}{2}$ pounds in 1 day being the maximum, the CO_2 capacity changed from 60.7 to 64.1 cc. per 100 cc. of plasma, the neutral urine became strongly alkaline, urinary CO_2 increased from 32 to 306 cc., and ammonia N decreased from 25 to 6 mg. in 100 cc. of urine. The large excess of base in alfalfa hay most efficiently overcame the slight acidosis indicated by the first sample.

The effect of eating grain only is seen in the second experimental period. There is a slight decrease in plasma CO_2 capacity. The urine has become less alkaline, CO_2 has dropped from 358 to 142 cc., ammonia has doubled in amount, and phosphorus has increased from 2.4 to 20.2 mg. in 100 cc. The effect of the excess of acid-forming elements in grain is here seen chiefly in the change in urinary composition.

In the last period, when corn silage was the sole food, evidences of increased acid production are seen at several points. The CO_2-combining power of plasma showed a decrease from 67.3 to 61.7 cc., the urinary CO_2 dropped from 385 to 200 cc., and the ammonia nearly doubled in amount. The behavior of the phosphorus fraction of the blood plasma in this set of experiments helps to clarify some of the apparent mysteries. It will be observed that the total phosphorus decreased nearly 16 per cent from October 16 to November 20. This change took place in both the inorganic and the lipoid fractions. Since these changes were found when the animal was on the same ration, some fundamental cause must be sought in explanation. The level of inorganic phosphorus in blood plasma has come to be regarded in this laboratory as a fairly reliable indication of an animal's nutritive condition. It was previously stated that the basal ration of silage and grain was inadequate for maintenance, and the decrease in phosphorus of the plasma is probably concrete evidence of this deficiency.

Some Effects of Fasting in the Cow.

The effects of fasting upon the composition of the blood and especially of the urine have been quite extensively investigated, particularly in the case of dogs and men. Such studies have contributed much of value to our knowledge of the metabolism of protein, fat, and carbohydrate, supplied solely from the subject's own body.

In order to gain a further understanding of the manner in which tissue neutrality in cattle is maintained, a fasting experiment of 7 days duration was carried through on a cow whose physical condition was excellent. This animal was neither pregnant nor lactating; her last calf was born 23 months prior to the beginning of the fast, and she had been dry for more than 13 months. These points are emphasized because of their probable bearing upon the course of the fast. It is evident that this cow had ample opportunity for building large reserves of tissue, and, what is doubtless of importance in this connection, of mineral constituents. During the experiment, the animal was kept in an ordinary stanchion and bedded with shavings. The fast may be considered complete, for she drank only 10 pounds of water throughout the fasting period of 7 days, although water was offered daily. The cow weighed 1,042 pounds at the beginning of the fast and 915 pounds when it closed, a loss representing more than 12 per cent of the original body weight.

Table V records the changes produced in blood plasma and urine by abstinence from food. Blood obtained about 5 hours after the last feeding of grain and silage on December 3 showed that 64.6 cc. of CO_2 were chemically bound by 100 cc. of plasma. This value, which is above the average of 61.5 cc. for cows living on mixed rations, shows that the animal was not acidotic and was in a good state for the subsequent fast. After 24 hours of fasting, the CO_2 capacity was 66.2 cc., indicating a slight increase in the alkaline reserve. The plasma CO_2 capacity remained at or near this latter figure throughout the period of inanition. Judging from the experience of Benedict (1915) in his investigation of the fasting man, Levanzin, who showed a decrease in the alveolar CO_2 tension on the 2nd fasting day and a further drop on the 14th day, one would have expected a lowering of the CO_2 capacity in

this case. It is plain that the CO_2 capacity of the plasma gave absolutely no indication of an acidotic condition.

The only evidence of acidosis afforded by the plasma is obtained from the phosphorus fraction. At the end of the 1st fasting day the inorganic phosphorus had increased about 11 per cent in amount. This is probably to be interpreted as an early mobili-

TABLE V.

Plasma and Urine of a Fasting Cow.

Date.	Day of fast.	Plasma.					Urine.				
		CO_2 capacity per 100 cc.	Ca per 100 cc.	Total P per 100 cc.	Inorganic P per 100 cc.	Lipoid P per 100 cc.	Specific gravity.	CO_2 per 100 cc.	NH_3 N per 100 cc.	Ca per 100 cc	P per 100 cc.
1919		*cc.*	*mg.*	*mg.*	*mg.*	*mg.*		*cc.*	*mg.*	*mg.*	*mg.*
Dec. 3	*	64.6	10.4	9.2	4.7	4.5	1.038	394	6	4.6	.16.1
" 4	1	66.2	10.0	10.5	5.2	5.3	1.032	359	7	3.2	20.6
" 5	2	67.2					1.033	79	10		
" 6	3	66.9	10.2	10.3	5.3	5.0	1.029	68	10	2.4	177.2
" 7	4	66.5					1.029	29	13		
" 8	5	65.5	10.0	9.7	5.2	4.5				2.2	217.2
" 9	6	66.4									
" 10	7	66.0	10.4	8.2	4.2	4.0	1.027			1.9	156.3

* The rations of this cow prior to the fast were:

		lbs.			*lbs.*		*lbs.*
From Nov. 23 to 29	Bran	6	Soy bean hay		2	Silage	24
Nov. 30	"	6	"	"	2	"	21
Dec. 1 to 2	"	6	"	"	2	"	18
" 3, a.m.	"	3				"	11

zation of the mineral reserves to assist in the maintenance of tissue neutrality. Inorganic phosphorus of the plasma remained at this higher level until the last day when it fell to a value lower than that obtained on the feeding day. In other short time fasting experiments of about 38 hours duration, conducted in this laboratory, larger increases in the phosphate of the plasma have been found; *i.e.*, 11, 24, and 81 per cent.[1]

[1] I am indebted to Dr. E. B. Meigs and Dr. C. A. Cary for permission to quote these unpublished results.

A change in the lipoid phosphorus of the plasma also occurred. After one day of inanition, there was an increase of about 18 per cent in this constituent. A gradual decrease in phosphatide phosphorus is seen throughout the remainder of the fast. The early augmentation of this fraction of the phosphorus content of the plasma is probably an expression of the quick mobilization of body fat, a phenomenon quite comparable to the increase observed in lactation, particularly in its earlier stages when there is a tendency for body fat to be mobilized and thrown into the blood stream, as suggested by Meigs, Blatherwick, and Cary (1919, *a*).

The calcium of the plasma showed small variations which lie within the limits of experimental error.

Turning our attention to the urine, the characteristic changes produced by lack of food are seen. The first urine obtained after the cow had eaten her last food gave the typical strongly alkaline reaction, with a large amount of CO_2 (394 cc)., small ammonia N (6 mg.), and a phosphorus content of 16.1 mg. in 100 cc. There was a slight change in the direction of decreasing alkalinity already evident at the end of the first fasting day, but this did not become well marked until the 2nd day, and on the 3rd day the CO_2 was 88 per cent less, ammonia 66 per cent more, and phosphorus ten times greater than in the original urine. The urine of the 4th day revealed a still further drop in CO_2 and an increase in ammonia. This latter sample was the first obtained by catheter, and the procedure unfortunately started a bladder condition which rendered determinations of CO_2 and ammonia of subsequent urines valueless. However, one sees that the elimination of acid had not yet reached its highest point, for the urine of the 5th day contained 217 mg. of phosphorus in 100 cc., as compared with 16 mg. in that of the control day. It would seem that the urinary elimination of phosphorus had reached its height about the 5th day, as the only remaining determination shows that this element had decreased to a value of 156 mg. in 100 cc. of the urine of the last day.

Experimental Acidosis in a Calf.

In Table VI are given the analytical data obtained from the plasma and urine of a calf which was rendered acidotic by feeding hydrochloric acid. This was one of the experiments planned to throw light on the hypothesis that plasma calcium is dependent

TABLE VI.

Experimental Acidosis in a Calf.

Date.	Plasma					Urine.			Remarks.
	CO_2 capacity per 100 cc.	Ca per 100 cc.	Total P per 100 cc.	Inorganic P per 100 cc.	Lipoid P per 100 cc.	Specific gravity.	CO_2 per 100 cc.	NH_3 N per 100 cc.	
1919	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*		*cc.*	*mg.*	
Aug. 29	68.3	12.1							2 days old.
Sept. 27	61.4	11.1	12.7	7.4	5.3	1.006	5.7	17	Up to date 12 lbs. of whole milk daily.
" 30						1.011	63.5	9	Sept. 28, 29, 30; 10 gm. of $NaHCO_3$ daily.
Oct. 2	66.4	11.2	13.8	8.2	5.6	1.005	51.7	4	Oct. 1; 20 gm. of $NaHCO_3$. Oct. 2, a.m.; 15 gm.
" 6						1.009	0.6	36	Oct. 3 and after; 180 cc. of 1 N HCl daily.
" 8	48.5	11.7	12.2	8.0	4.2	1.010	0.2	67	Oct. 6; 180 cc. of 1 N HCl. Oct. 7; 240 cc. Oct. 8, a.m.; 180 cc. P in urine of Oct. 8, 75 mg. per 100 cc.

upon the bicarbonate content of this fluid. At 2 days of age the animal showed a CO_2 capacity of 68.3 cc. and a calcium content of 12.1 mg. per 100 cc. of plasma. About 1 month later these values had become 61.4 cc. and 11.1 mg., respectively. After this sample of blood was taken on September 27, 10 gm. of $NaHCO_3$

were added to the feed of 12 pounds of whole milk daily. On October 1, 20 gm. of $NaHCO_3$ were given and on the following morning it received 15 gm. of this substance. Blood taken on this date revealed the characteristic increase in CO_2 capacity. The urine was alkaline, its CO_2 had increased from 6 to 52 cc., while the ammonia decreased from 17 to 4 mg. of N per 100 cc. The calf was immediately changed to an acid diet, receiving with milk 180 cc. of 1 N HCl daily from October 3 to 6 inclusive. On the following day the dose was increased to 240 cc., and on the morning of the 8th it drank 180 cc. of this acid. A sample of blood obtained on this latter date showed that the plasma CO_2 capacity had responded with the well known drop to 48.5 cc. The corresponding urine was decidedly acid, its CO_2 had practically disappeared, and the ammonia had increased to 67 mg. of N in 100 cc.

Regarding the hypothetical relation between plasma calcium and bicarbonate this experiment offers slight positive evidence. A decrease in CO_2 capacity from 61.4 to 48.5 cc. was accompanied by an increase in calcium from 11.1 to 11.7 mg. per 100 cc. of plasma. However, another experiment on a calf 2 weeks of age resulted in a decrease of calcium from 11.5 to 11.0 mg. when the CO_2 capacity was lowered from 73.0 to 54.1 cc. by feeding hydrochloric acid. These findings indicate that the level of plasma calcium is very little or not at all influenced by physiological changes in the alkaline reserve.

Some Effects of Feeding Benzoic Acid to a Calf.

One of the striking differences in the metabolism of calves and cows is found in the small excretion of hippuric acid in the urine of young animals. It is only when the latter begin to eat foods other than milk that appreciable amounts of this acid appear. Among the foods consumed by herbivora which give rise to the formation of hippuric acid, meadow hay was observed by Weiske (1876) to be the most efficient. This author believed that most of the hippuric acid precursor was found in the weeds which contaminated the hay. He also observed that wheat and oat straws lead to the formation of considerable amounts of hippuric acid, but none of this acid was excreted when the experimental animals,

wethers, were fed pea straw, bean straw, unpeeled potatoes, peas, linseed, wheat, or oats.

It seemed to be an interesting point to discover how a young calf would react to benzoic acid when fed along with a non-hippuric acid-forming diet. A calf, aged 1 month, was accordingly placed upon a uniform milk diet to which a certain amount of benzoic acid was added. On February 16, samples of blood and urine resulting from the milk diet were obtained. Normal values for a calf of that age are shown in Table VII. Plasma CO_2 capac-

TABLE VII.

Composition of Blood and Urine of Calf Fed Benzoic Acid.

Date.	Plasma.					Urine.				Remarks.
	CO_2 capacity per 100 cc.	Ca per 100 cc.	Total P per 100 cc.	Inorganic P per 100 cc.	Lipoid P per 100 cc.	Specific gravity.	CO_2 per 100 cc.	NH_3 N per 100 cc.	P per 100 cc.	
1920	*cc.*	*mg.*	*mg.*	*mg.*	*mg.*		*cc.*	*mg.*	*mg.*	
Feb. 16	71.0	10.5	10.3	7.5	2.8	1.007	9.7	14.6	47.3	Whole milk diet. 5 gm. of benzoic, p.m.
" 17						1.012	1.1	39.3		10 gm. of benzoic.
" 18	68.1	9.9	10.4	7.6	2.8	1.015	1.4	71.6	142.1	5 gm. of benzoic, a.m., 5 gm. of benzoic, p.m.
" 19						1.008	2.5	30.5		5 gm. of benzoic, a.m., 10 gm. of benzoic, p.m.
" 20	71.1	10.4	10.4	7.7	2.7	1.008	1.6	26.7	58.7	10 gm. of benzoic, a.m.

ity, 71.0 cc., was somewhat above the higher adult figures. The urine was neutral in reaction and contained 9.7 cc. of CO_2, 14.6 mg. of ammonia N, and 47.3 mg. of P in 100 cc. Urine obtained the following day after the calf had received 10 gm. of benzoic acid was acid to litmus, and contained only 1.4 cc. of CO_2 in 100 cc., and the ammonia had nearly tripled in value. After having received a total of 20 gm. of the acid, the plasma CO_2 capacity of the 2nd day decreased slightly to 68.0 cc. per 100 cc. The corresponding urine was decidedly acid, CO_2 was the same as on the preceding day, ammonia N increased to 71.6 mg., and phos-

phorus was three times larger than in the first sample. The results thus far indicated the possibility of producing an acidosis somewhat comparable to that resulting from the use of mineral acids. It was, therefore, decided to push the dosage of the acid. Urine obtained on the following morning gave values for CO_2 and ammonia which pointed to a return to normal conditions. On the afternoon of February 19, the dose was increased to 10 gm. In spite of this larger amount of acid, samples of blood and urine secured the next morning revealed values for plasma CO_2 capacity, urinary ammonia, and phosphorus which were nearly comparable with those obtained before the administration of benzoic acid.

It is evident that a condition of acidosis was produced when benzoic acid was first administered, but that nearly complete recovery followed in spite of larger doses of the acid. During the period of acidosis, the calf was suffering from diarrhea, but the feces were normal in appearance on the last day of the experiment. How this digestive disturbance may have influenced the results is not known. Determination of the hippuric and benzoic acid content of the blood and urine would, no doubt, have aided in the interpretation of the experimental results.

DISCUSSION.

The experiments dealing with changes in blood and urine resulting from variations in diet leave no question, it seems to me, that the ability of plasma to bind carbon dioxide chemically may be altered by the ingestion of foods of different potential acidity or alkalinity. This is very clearly shown in the case of Cow 56, Table III. The CO_2 capacity of her plasma, after being fed solely on an acid-forming grain mixture was 58.6 cc. in contrast with a value of 69.2 cc. when she ate the strongly base-forming alfalfa hay. Similar responses of the plasma alkaline reserve to changes in diet, although less marked, are evident in the case of Cow 66, Table IV.

The ability of a diet of corn silage, the ash of which contains an excess of basic elements, to decrease plasma CO_2 capacity and to cause the formation of acid urines is a most interesting phenomenon. This effect is definitely shown in the case of Cow 56, Table

III. After she had eaten heartily of this food, CO_2 chemically bound amounted to 54.8 cc. per 100 cc. of plasma. The corresponding urine was decidedly acid to litmus, and contained only 4 cc. of CO_2 in 100 cc., together with considerable quantities of ammonia and phosphorus. The reserve alkalinity of this animal was certainly reduced as the result of eating a food which is generally considered base-forming in character.

How may this unexpected result be explained? May not the organic acids of corn silage be responsible for this anomalous effect? According to Dox and Neidig (1912, 1913), 100 gm. of dried corn silage contain about 1.63 gm. of volatile acids of which acetic acid comprises 90 per cent. The chief remaining volatile acid is propionic. Butyric acid occurs in small amounts in good material and increases when spoilage occurs. The non-volatile acid of silage is racemic lactic which is present in greater amounts than all other acids combined. The proportion of non-volatile to volatile acids is approximately 100:75. If a calculation is made of the amount of organic acids in the silage consumed by Cow 56 in her last three meals before blood and urine samples were taken, it is found that she received about 373 gm. of organic acids distributed as follows: acetic 144 gm., other volatile acids 16 gm., and racemic lactic 213 gm. Of this total, slightly more than one-third was eaten the morning that samples were taken. Cow 66 during the corresponding period ate $62\frac{1}{2}$ pounds of silage which would yield nearly as large an intake of organic acids (327 gm.). However, she ingested but $4\frac{1}{2}$ pounds of silage on the morning that samples of blood and urine were obtained. The changes in her blood and urine though distinct were not nearly so marked as in the other case.

Further evidence bearing upon the point is the following: Cow 66 possessed a lower CO_2 capacity of the plasma; her urine contained less CO_2 and more ammonia when she ate silage alone than when smaller amounts of silage (16 pounds) with the addition of 2 pounds of grain, an acid-forming food, were consumed. A reasonable explanation of this phenomenon is made by assuming that a cow is able to oxidize certain amounts of the organic acids of silage, but when this limit is reached these acids act like a mineral acid in lowering the alkaline reserve and causing the elimination of acid urines. In the present state of our know-

ledge concerning the quantities of organic acids which the body is able to oxidize, one cannot hazard an opinion as to whether one or more of the acids found in silage are responsible for the effects observed. It is also probable that an increased formation of hippuric acid may be a factor contributing to this greater acid production.

Those authors who have been unable to change the alkaline reserve by altering the diet were probably unfortunate in their selection of experimental subjects, or the food consumption may not have been sufficiently great to produce the effect.

Further study of the calcium content of the blood plasma of cattle confirmed the observations previously reported from this laboratory. The plasma of young calves contains more of this element than does that of cows. In adult animals the level of plasma calcium tends to remain quite uniform but it may be altered somewhat by appropriate changes in the amount supplied with the rations. Experiments designed to throw light on the hypothesis that the amount of calcium in plasma is dependent upon the bicarbonate content of this fluid failed to demonstrate any such relation when the changes in bicarbonate were within physiological limits. These results are not entirely in harmony with those of Allers and Bondi (1907) who state that they were able to double the calcium content of the blood of rabbits by feeding hydrochloric acid. Their doses were proportionally much larger than mine, and it seems probable that feeding acid has no effect on plasma calcium unless sufficiently large amounts are given to produce a great depletion in the alkaline reserve.

In cattle the regulation of tissue neutrality assumes a different character than in omnivora or carnivora. The primary concern of the cow is to rid the body of excess base which is normally so abundantly furnished by the food. The problem, therefore, is generally quite the opposite of that which relates to non-herbivorous animals. Accordingly one finds the cow excreting a strongly alkaline urine which contains large amounts of carbonate, equivalent in many instances to about 40 gm. of CO_2 daily. Only small quantities of ammonia and phosphorus are found owing to small need for the elimination of acid. When the natural inclinations for food are interfered with by restricting the animal to an acid-forming food, by fasting, or by feeding acid *per se*, an

approach to the conditions obtaining in man or carnivora is manifest. Under these circumstances the body clings to its alkali, as indicated by a decreased CO_2 output in the urine. Likewise, increased amounts of urinary ammonia and phosphorus appear in response to the necessity for increased acid excretion.

Calves, on the other hand, approach more nearly the conditions which obtain in man. While calves live on an approximately neutral diet of milk, their urines are normally neutral in reaction, and contain small amounts of carbonate, and significantly greater quantities of ammonia and phosphorus. In spite of these differences in the urines of young and adults, the carbon dioxide-combining power of the plasma of calves is decidedly higher than that of cows. It is clear that a fundamentally different regulatory mechanism is functioning in the two cases. A comparison of the hydrogen ion concentration of the two bloods, and a study of the respiration of young and adults should prove valuable aids to a further elucidation of this problem.

Study of the carbon dioxide capacity of cow plasma shows that a determination of this value is of little aid in detecting mildly acidotic conditions. Although the variations in the ammonia content of urine are significant, many complicating factors enter to make an estimation of this constituent not entirely suitable for an indicator of increased acid production. Apparently, the most reliable indication of acidosis in cattle is found in the output of carbonate in the urine. Decreased amounts of urinary CO_2 are the first expression by the cow of an increased acid metabolism. This reaction is quickly evident, whereas rather forced conditions must prevail before large changes in plasma CO_2 capacity are produced.

SUMMARY.

The alkaline reserve of the cow, as measured by the carbon dioxide-combining power of the plasma, is remarkably constant. Twenty-two determinations of the plasma CO_2 capacity of sixteen individuals gave an average value of 61.5 cc. per 100 cc. with maximum and minimum figures of 68.3 and 55.1 cc., respectively.

The amount of CO_2 chemically bound by the plasma of young calves is decidedly greater than by that of adult animals. The average CO_2 capacity of the plasma of seven calves ranging in

age from 2 to 14 days was 73.0 cc. Maximum and minimum values for the same calves were 80.6 and 68.3 cc. per 100 cc.

Significant changes in the alkaline reserve of cows were produced by altering the diet with respect to the acidity or alkalinity of its ash. Corn silage, a food generally considered to be base-forming in character, when fed in large amounts to a cow decreased the plasma CO_2 capacity and led to the formation of an acid urine. Reasons were advanced for believing that this anomalous result was due to the incomplete oxidation of the organic acids of silage.

A cow that was fasted for 7 days failed to show any decrease in plasma CO_2 capacity. An increase in the inorganic phosphorus of the plasma was interpreted as an expression of the mobilization of the mineral reserves to aid in maintaining tissue neutrality.

The amount of calcium in the plasma of cattle may be influenced by the quantities in the ration but the changes are not great even when large variations are made in the intake. Under physiological conditions, the level of plasma calcium appears to be very little, if at all, dependent upon the bicarbonate content of this fluid.

Comment was made upon some of the differences between herbivora and carnivora or omnivora with respect to the maintenance of tissue neutrality. In this connection, the practicability of measuring the CO_2 content of the urine of herbivora to determine changes in the excretion of base was emphasized.

The writer desires to express his thanks to Dr. E. B. Meigs for his friendly criticism, and to Mr. T. E. Woodward and Mr. H. T. Converse for assistance with the experimental animals.

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EFFECT OF ANESTHETICS ON VARIOUS CELL ACTIVITIES.

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Experiments on the Aquatic Plant, Elodea.

In order to determine CO_2 content of the medium from the pH, the solution used in the experiments was 0.0025 N $NaHCO_3$, brought first to pH 7.6 by adjusting the CO_2 content. A change in pH of 0.1 indicated a change in CO_2 content of 1.5 cc. per liter. The pH was determined by comparison with a set of standards prepared from Sörensen's phosphate mixtures, colored with phenolsulfonephthalein, and sealed in Nonsol test-tubes 25 mm. in diameter.

For oxygen determinations, a terminal sprig of *Elodea*, 8 inches long, was washed in the bicarbonate solution, placed in a 100 cc. bottle filled with the same solution, and the bottle enclosed in an opaque container sunk in the thermostat. The feathery nature of this plant facilitates exchange with the medium. At the same time another 100 cc. bottle was filled with the solution without the *Elodea*. At the end of 3 hours the plant was pulled out with forceps and the solutions in the two bottles were titrated by the Winkler method. The difference in the two titrations gave the O_2 consumption. The same sprig of *Elodea* was employed in a similar manner for determination of O_2 consumption in the bicarbonate solution to which the anesthetic had been added. A second sprig of the plant was used to determine change in pH and exosmosis, the contents of the 100 cc. bottle being used to fill the tubes for the two determinations. The exosmosis of chlorides was determined with a Richard nephel-

ometer. Photosynthesis was determined similarly to respiration except that the opaque container was omitted and a 240 watt stereopticon lamp was placed 1 foot from the plant. The heat of the lamp was screened off by a layer of water. Protoplasmic rotation was determined by means of a micrometer eyepiece in a microscope.

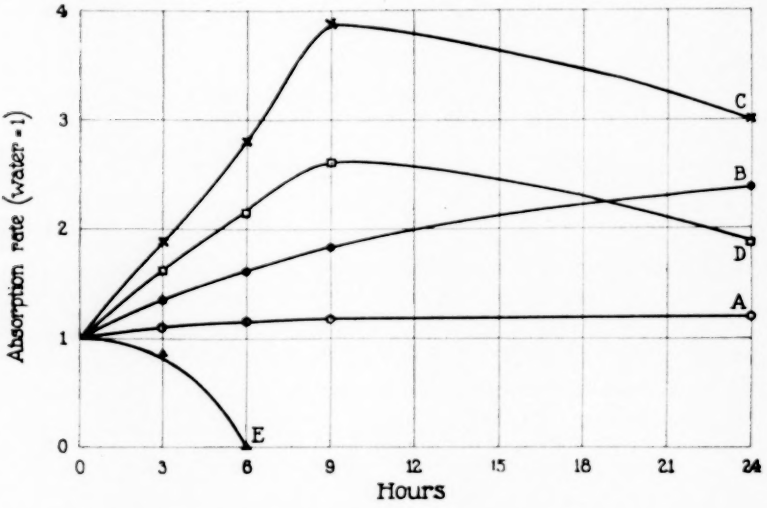

FIG. 1. Relative rate of O_2 absorption of *Elodea* in solutions of alcohol. Curve A, 1 per cent; B, 1.5 per cent; C, 3 per cent; D, 6 per cent; E, 15 per cent (Table I).

The experiments were made in a thermostat at 30°, the best record of which showed a fluctuation of 0.01° in 1 day and a continuous deviation amounting to 0.1° in 10 days. Since a variation of 1° means an error of 25 per cent, it was necessary that respiration with and without the anesthetic be carried on at the same temperature.

A 1 per cent solution of alcohol (Fig. 1, Curve A) produced practically no effect on rate of O_2 consumption. Even after 3 days the rate showed no decrease (1.21). In the 1.5 per cent solution (Curve B) the rate showed a steady increase for 24 hours. When such a piece was kept in the alcohol 2 days longer,

the rate remained practically the same as at the close of 24 hours. If it was returned to water, the rate seemed to increase still more rapidly, and to continue so for at least 3 days. In one instance the rate after 3 days in tap water following 24 hours in 1.5 per cent alcohol was 2.86. This is in harmony with the fact that slight anesthetization hastens the opening of dormant buds on deciduous plants.

In the 3 per cent solution (Curve C) the rate increased still more rapidly, reaching 3.87 at the close of 9 hours, but by the end of 24 hours had decreased to 2.99 (Table I, No. 4). In 6 per cent solution (Curve D) the increase was not so great, and the point reached at the close of 24 hours was correspondingly lower. In the 15 per cent solution (Curve E) there was no increase. Since there existed the possibility that an increase may have occurred, lasting for a much briefer period than that employed in this instance, the experiment was repeated and determinations were made after intervals of 20 minutes (Table II).

No increase of O_2 consumption was discernible even when the periods were reduced to 20 minutes. The experiment was repeated using 10 minute intervals, but, although the differences were so slight as to be inconclusive, they indicated that no decrease of rate was occurring.

The curves obtained from measurement of changes of pH due to CO_2 were very similar to those of O_2 consumption in solutions of low concentration (alcohol 1, 1.5, and 3 per cent, Table III and Fig. 2). But 6 and 15 per cent alcohol caused acceleration of rate at first, the latter producing the maximum change.

In all the solutions of alcohol, except the 1.0 per cent solution, the rate of exosmosis of chlorides from the cell increased (Table IV and Fig. 3). This acceleration was very slight, however, in all cases except the 6 and 15 per cent solutions. It was especially marked in the latter where the cells were so injured that after 15 minutes immersion irreversible plasmolysis occurred.

In the 15 per cent solution protoplasmic rotation had ceased entirely (Table V and Fig. 4) within 1 minute, and after 15 minutes was irreversible. In the 6 per cent solution the decrease in rate was gradual; in the 3 and 1.5 per cent solutions an increase was shown. If these tips of *Elodea* were returned to water after

TABLE I.

Respiration as Measured by the Amount of Oxygen Extracted by Elodea from Solutions.

9 hrs. in water and 24 hrs. in solution. Readings taken after 3 hr. periods in water, and in anesthetics at the close of 3, 6, and 9 hrs. immersion, and after the period extending from 21 to 24 hrs. At the beginning of each 3 hr. period, the plant was placed in a fresh solution. For corresponding curves see Figs. 1, 5, 10, and 14.

Plant No.	Solution.	Concentration.	Time.	Thiosulfate.	Change.	Relative rate of O_2 absorption.
		per cent	*hrs.*	*cc.*	*cc.*	
1	Water.		1–3	2.25–1.83	0.42	
	"		3–6	2.25–1.84	0.41	
	"		6–9	2.25–1.83	0.42	
	"		21–24	2.25–1.83	0.42	
2	"		1–3	2.25–1.85	0.40	
	"		3–6	2.25–1.85	0.40	
	"		6–9	2.25–1.85	0.40	
	Alcohol.	1	1–3	2.25–1.80	0.45	1.12
	"	1	3–6	2.25–1.78	0.47	1.16
	"	1	6–9	2.25–1.78	0.47	1.16
	"	1	21–24	2.25–1.77	0.48	1.20
3	Water.		1–3	2.25–1.85	0.40	
	"		3–6	2.25–1.85	0.40	
	"		6–9	2.25–1.85	0.40	
	Alcohol.	1.5	1–3	2.25–1.71	0.54	1.350
	"	1.5	3–6	2.25–1.61	0.64	1.600
	"	1.5	6–9	2.25–1.52	0.73	1.825
	"	1.5	21–24	2.25–1.30	0.95	2.375
4	Water.		1–3	2.25–1.80	0.45	
	"		3–6	2.25–1.80	0.45	
	"		6–9	2.25–1.79	0.46	
	Alcohol.	3	1–3	2.25–1.40	0.85	1.89
	"	3	3–6	2.25–0.99	1.26	2.80
	"	3	6–9	2.25–0.51	1.74	3.87
	"	3	21–24	2.25–0.91	1.34	2.99
5	Water.		1–3	2.25–1.85	0.40	
	"		3–6	2.25–1.85	0.40	
	"		6–9	2.25–1.85	0.40	
	Alcohol.	6	1–3	2.25–1.60	0.65	1.63
	"	6	3–6	2.25–1.39	0.86	2.15
	"	6	6–9	2.25–1.20	1.05	2.63
	"	6	21–24	2.25–1.50	0.75	1.88

TABLE I—*Continued.*

Plant No.	Solution.	Concentration.	Time.	Thiosulfate.	Change.	Relative rate of O_2 absorption.
		per cent	*hrs.*	*cc.*	*cc.*	
6	Water.		1–3	2.25–1.83	0.42	
	"		3–6	2.25–1.83	0.42	
	"		6–9	2.25–1.83	0.42	
	Alcohol.	15	1–3	2.25–1.90	0.35	0.83
	"	15	3–6	2.25–2.25	0.00	0.00
7	Water.		1–3	2.25–1.75	0.50	
	"		3–6	2.25–1.77	0.48	
	"		6–9	2.25–1.75	0.50	
	Ether.	1.5	1–3	2.25–1.50	0.75	1.50
	"	1.5	3–6	2.25–0.80	1.45	2.90
	"	1.5	6–9	2.25–1.45	0.90	1.80
	"	1.5	21–24	2.25–1.60	0.65	1.30
8	Water.		1–3	2.25–1.52	0.73	
	"		3–6	2.25–1.50	0.75	
	"		6–9	2.25–1.50	0.75	
	Ether.	3	1–3	2.25–0.95	1.40	1.86
	"	3	3–6	2.25–1.15	1.10	1.46
	"	3	6–9	2.25–1.55	0.70	0.93
	"	3	21–24	2.25–2.25	0.00	0.00
9	Water.		1–3	2.25–1.60	0.65	
	"		3–6	2.25–1.60	0.65	
	"		6–9	2.25–1.60	0.65	
	Ether.	5	1–3	2.22–1.12	1.10	1.69
	"	5	3–6	2.22–1.44	0.78	1.20
	"	5	6–9	2.22–1.98	0.24	0.37
	"	5	21–24	2.22–2.22	0.00	0.00
10	Water.		1–3	2.25–1.55	0.70	
	"		3–6	2.25–1.55	0.70	
	"		6–9	2.25–1.55	0.70	
	Ether.	8	1–3	2.17–1.27	0.90	1.29
	"	8	3–6	2.17–2.17	0.00	0.00
11	Water.		1–3	2.25–1.90	0.35	
	"		3–6	2.25–1.91	0.34	
	"		6–9	2.25–1.91	0.34	
	Chloroform.	0.05	1–3	2.25–1.85	0.40	1.13
	"	0.05	3–6	2.25–1.70	0.55	1.57
	"	0.05	6–9	2.25–1.60	0.65	1.86
	"	0.05	21–24	2.25–1.40	0.85	2.43

TABLE I—*Concluded.*

Plant No.	Solution.	Concentration.	Time.	Thiosulfate.	Change.	Relative rate of O_2 absorption.
		per cent	*hrs.*	*cc.*	*cc.*	
12	Water.		1–3	2.25–1.80	0.45	
	"		3–6	2.25–1.80	0.45	
	"		6–9	2.25–1.79	0.46	
	Chloroform.	0.15	1–3	2.25–1.50	0.75	1.63
	"	0.15	3–6	2.25–1.70	0.55	1.20
	"	0.15	6–9	2.25–1.90	0.35	0.77
	"	0.15	21–24	2.25–2.05	0.20	0.44
13	Water.		1–3	2.25–1.85	0.40	
	"		3–6	2.25–1.84	0.41	
	"		6–9	2.25–1.85	0.40	
	Chloroform.	0.30	1–3	2.24–1.69	0.55	1.38
	"	0.30	3–6	2.24–2.24	0.00	0.00
14	Water.		1–3	2.25–1.95	0.30	
	"		3–6	2.25–1.95	0.30	
	"		6–9	2.25–1.95	0.30	
	Chloretone.	0.05	1–3	2.25–1.90	0.35	1.17
	"	0.05	3–6	2.25–1.70	0.55	1.83
	"	0.05	6–9	2.25–1.55	0.70	2.33
	"	0.05	21–24	2.25–1.40	0.85	2.83
15	Water.		1–3	2.25–1.85	0.40	
	"		3–6	2.25–1.84	0.41	
	"		6–9	2.25–1.85	0.40	
	Chloretone.	0.1	1–3	2.25–2.05	0.20	0.50
	"	0.1	3–6	2.25–2.15−	0.10+	0.25+
	"	0.1	6–9	2.25–2.15	0.10	0.25
	"	0.1	21–24	2.25–2.15+	0.10−	0.25−

TABLE II.

Oxygen Consumption by Elodea in 15 Per Cent Alcohol during Six 20 Min. Periods.

Solution.	Thiosulfate.	Change	Relative rate of O_2 absorption.
	cc.	*cc.*	
Water.	2.15–1.90	0.25	
Alcohol.	2.15–1.93	0.22	0.88
"	2.15–2.03	0.12	0.48
"	2.15–2.10	0.05	0.20
"	2.15–2.15	0.00	0.00
"	2.15–2.14	0.01(?)	0.04(?)
"	2.15–2.15	0.00	0.00

TABLE III.

CO_2 Liberated as Measured by the Changes in pH of Solutions.

Plant immersed 9 hrs. in water and 24 hrs. in solution. Readings taken after 3 hr. periods in water, and in anesthetics at the close of 3, 6, and 9 hrs. immersion, and after the period extending from 21 to 24 hrs. At the beginning of each 3 hr. period, the plant was placed in a fresh solution. For corresponding curves, see Figs. 2, 6, 11, and 15.

Plant No.	Solution.	Concentration.	Time.	pH	Change.	Relative rate of CO_2 elimination.
		per cent	*hrs.*		*cc.*	
2	Water.		1–3	7.8 –7.6	0.20	
	"		3–6	7.8 –7.6	0.20	
	"		6–9	7.8 –7.6	0.20	
	Alcohol.	1	1–3	7.6 –7.4–	0.20+	1.00+
	"	1	3–6	7.6 –7.4–	0.20+	1.00+
	"	1	6–9	7.6 –7.4–	0.20+	1.00+
	"	1	21–24	7.6 –7.35	0.25	1.25
3	Water.		1–3	7.8 –7.6	0.20	
	"		3–6	7.8 –7.6	0.20	
	"		6–	7.8 –7.6+	0.20–	
	Alcohol.	1.5	1–3	7.6 –7.35	0.25	1.25
	"	1.5	3–6	7.6 –7.3	0.30	1.50
	"	1.5	6–9	7.6 –7.3	0.30	1.50
	"	1.5	21–24	7.6 –7.2	0.40	2.00
4	Water.		1–3	7.8 –7.55	0.25	
	"		3–6	7.8 –7.55	0.25	
	"		6–9	7.8 –7.55	0.25	
	Alcohol.	3	1–3	7.6 –7.25	0.35	1.4
	"	3	3–6	7.6 –7.20	0.40	1.6
	"	3	6–9	7.6 –7.20	0.40	1.6
	"	3	21–24	7.6 –7.25	0.35	1.4
5	Water.		1–3	7.8 –7.6	0.20	
	"		3–6	7.8 –7.6	0.20	
	"		6–9	7.8 –7.6	0.20	
	Alcohol.	6	1–3	7.6 –7.25	0.35	1.75
	"	6	3–6	7.6 –7.25	0.35	1.75
	"	6	6–9	7.6 –7.3	0.30	1.50
	"	6	21–24	7.6 –7.4	0.20	1.00
6	Water.		1–3	7.8 –7.6	0.20	
	"		3–6	7.8 –7.6	0.20	
	"		6–9	7.8 –7.6	0.20	
	Alcohol.	15	1–3	7.6 –7.2	0.40	2.0
	"	15	3–6	7.6 –7.4	0.20	1.0
	"	15	6–9	7.6 –7.6	0.00	0.0

TABLE III—*Continued.*

Plant No.	Solution.	Concentration.	Time.	pH	Change.	Relative rate of CO_2 elimination.
		per cent			*cc.*	
7	Water.		1–3	7.8 –7.5	0.30	
	"		3–6	7.8 –7.5	0.30	
	"		6–9	7.8 –7.5	0.30	
	Ether.	1.5	1–3	7.8 –7.45	0.35	1.17
	"	1.5	3–6	7.8 –7.4	0.40	1.33
	"	1.5	6–9	7.8 –7.4	0.40	1.33
	"	1.5	21–24	7.8 –7.4	0.40	1.33
8	Water.		1–3	7.8 –7.5	0.30	
	"		3–6	7.8 –7.5	0.30	
	"		6–9	7.8 –7.5	0.30	
	Ether.	3	1–3	7.8 –7.4	0.40	1.33
	"	3	3–6	7.8 –7.6	0.20	0.67
	"	3	6–9	7.8 –7.7	0.10	0.33
	"	3	21–24	7.8 –7.8	0.00	0.00
9	Water.		1–3	7.8 –7.5	0.30	
	"		3–6	7.8 –7.5	0.30	
	"		6–9	7.8 –7.5	0.30	
	Ether.	5	1–3	7.8 –7.25	0.40	1.83
	"	5	3–5	7.8 –7.8	0.00	0.00
10	Water.		1–3	7.8 –7.4	0.40	
	"		3–6	7.8 –7.4	0.40	
	"		6–9	7.8 –7.4	0.40	
	Ether.	8	1–3	7.7 –6.8	0.90	2.25
	"	8	3–6	7.7 –7.7	0.00	0.00
11	Water.		1–3	7.8 –7.6	0.20	
	"		3–6	7.8 –7.6	0.20	
	"		6–9	7.8 –7.6	0.20	
	Chloroform.	0.05	1–3	7.5 –7.3	0.20	1.0
	"	0.05	3–6	7.5 –7.3	0.20	1.0
	"	0.05	6–9	7.5 –7.25	0.25	1.25
	"	0.05	21–24	7.5 –7.2	0.30	1.50
12	Water.		1–3	7.8 –7.55	0.25	
	"		3–6	7.8 –7.55	0.25	
	"		6–9	7.8 –7.55	0.25	
	Chloroform.	0.15	1–3	7.7 –7.2	0.50	2.0
	"	0.15	3–6	7.7 –7.45	0.25	1.0
	"	0.15	6–9	7.7 –7.45	0.25	1.0
	"	0.15	21–24	7.7 –7.6+	0.10–	0.4–

TABLE III—*Concluded.*

Plant No.	Solution.	Concentration.	Time.	pH	Change.	Relative rate of CO_2 elimination.
		per cent	*hrs.*		*cc.*	
13	Water.		1–3	7.8 –7.6	0.20	
	"		3–6	7.8 –7.6	0.20	
	"		6–9	7.8 –7.6	0.20	
	Chloroform.	0.3	1–3	7.8– 7.2	0.60	3.0
	"	0.3	3–6	7.8 –7.4	0.40	2.0
	"	0.3	6–9	7.8 –7.6	0.20	1.0
	"	0.3	21–24	7.8 –7.8	0.00	
14	Water.		1–3	7.8 –7.6	0.20	
	"		3–6	7.8 –7.6	0.20	
	"		6–9	7.8 –7.6	0.20	
	Chloretone.	0.05	1–3	7.55–7.25	0.30	1.50
	"	0.05	3–6	7.55–7.15	0.40	2.00
	"	0.05	6–9	7.55–7.1	0.45	2.25
	"	0.05	21–24	7.55–6.95	0.60	3.00
15	Water.		1–3	7.8 –7.4	0.4	
	"		3–6	7.8 –7.4	0.4	
	"		6–9	7.8 –7.4	0.4	
	Chloretone.	0.1	1–3	7.65–7.00	0.65	1.62
	"	0.1	3–6	7.6 –6.85	0.8	2.00
	"	0.1	6–9	7.6 –6.95	0.7	1.75
	"	0.1	21–24	7.6 – .35	0.3	0.75

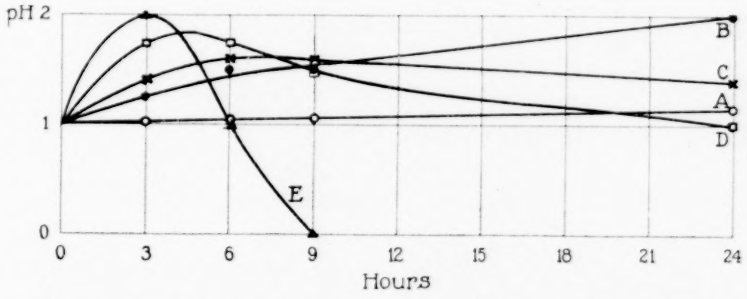

FIG. 2. Relative change of pH of solutions of alcohol in which *Elodea* had been kept. Curve A, 1 per cent; B, 1.5 per cent; C, 3 per cent; D, 6 per cent; E, 15 per cent (Table III).

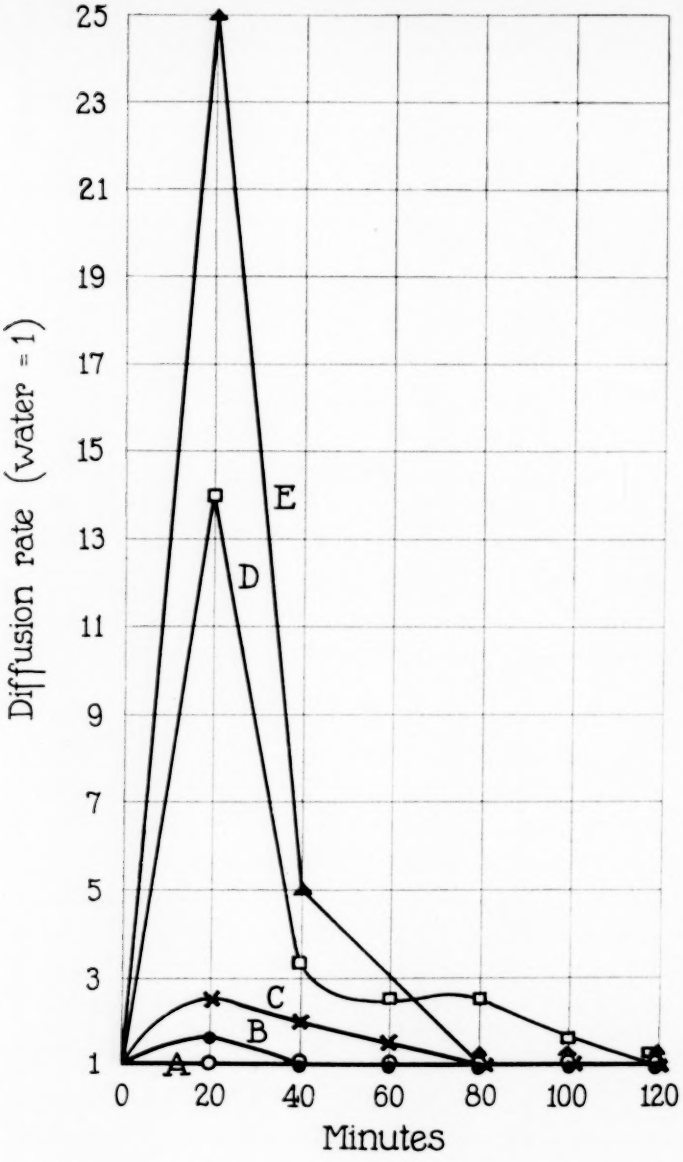

FIG. 3. Diffusion of chlorides from *Elodea* in solutions of alcohol during six 20 minute periods. Curve A, 1 per cent; B, 1.5 per cent; C, 3 per cent; D, 6 per cent; E, 15 per cent.

TABLE IV.

Rate of Diffusion of Chlorides from Elodea during Six 20 Min. Periods.

For corresponding curves, see Figs. 3, 7, 12, and 16.

Solution.	Concentration.	Diffusion by periods, 20 min. each.					
		1	2	3	4	5	6
	per cent						
Alcohol.	1.0	1.0	1.0	1.0	1.0	1.00	1.0
"	1.5	1.6	1.0	1.0	1.0	1.00	1.0
"	3.0	2.5	2.0	1.5	1.0	1.00	1.0
"	6.0	14.0	3.3	2.5	2.5	1.67	1.0
"	15.0	25.0	5.0	3.0	1.0	1.00	1.0
Ether.	1.5	1.75	1.00	1.00	1.00	1.0	1.00
"	3.0	1.67	2.50	3.33	3.33	2.0	1.67
"	5.0	20.00	3.33	2.80	2.67	2.5	2.50
"	8.0	25.00	5.00	3.00	1.00	1.0	1.00
Chloroform.	0.05	3.0	3.0	3.0	3.0	3.0	3.0
"	0.15	10.0	4.0	3.0	2.5	2.0	1.5
"	0.30	20.0	5.0	1.0	1.0	1.0	1.0
Chloretone.	0.05	1.5	1.0	1.0	1.0	1.0	1.0
"	0.10	5.0	2.0	1.0	1.0	1.0	1.0

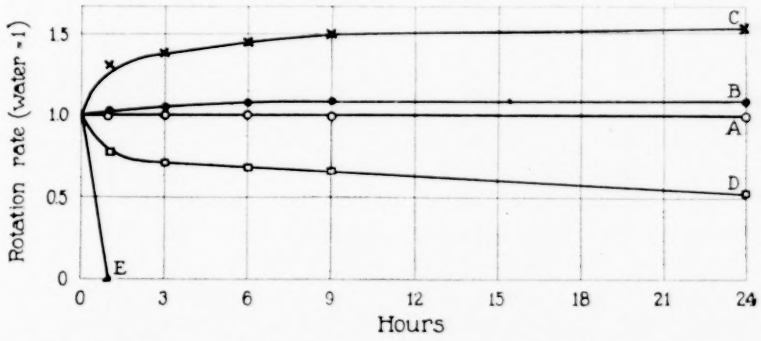

FIG. 4. Rate of protoplasmic rotation in *Elodea* in solutions of alcohol. Curve A, 1 per cent; B, 1.5 per cent; C, 3 per cent; D, 6 per cent; E, 15 per cent.

immersion for 24 hours, the rate increased still more and this more rapid rotation was continued for several days.

Since the rate of photosynthesis was normally twelve times that of respiration, the changes in rate of respiration may be omitted in determining photosynthesis without vitiating the

TABLE V.

Rate of Rotation of Elodea in Solutions of Alcohol, Ether, Chloroform, and Chloretone.

Compared with rate in water taken as the unit. For curves, see Figs. 4, 8, 13, and 17.

Solution.	Concentration.	Rotation in water.	Rate of rotation in solutions.				
			1	3	6	9	24
	per cent						
Alcohol.	1.0	1.0	0.99	1.00	1.00	1.00	0.99
"	1.5	1.0	1.02	1.05	1.07	1.08	1.09
"	3.0	1.0	1.31	1.35	1.43	1.49	1.53
"	6.0	1.0	0.76	0.71	0.67	0.67	0.52
"	15.0	1.0	0.00	0.00	0.00	0.00	0.00
Ether.	1.5	1.0	0.99	0.90	0.92	0.89	0.57
"	3.0	1.0	0.59	0.30	0.28	0.29	0.22
"	5.0	1.0	0.00	0.00	0.00	0.00	0.00
"	8.0	1.0	0.00	0.00	0.00	0.00	0.00
Chloroform.	0.05	1.0	1.17	1.30	1.31	1.34	1.36
"	0.15	1.0	0.74	0.65	0.57	0.52	0.00
"	0.30	1.0	0.00	0.00	0.00	0.00	0.00
Chloretone.	0.05	1.0	0.88	0.71	0.63	0.60	0.00
"	0.10	1.0	0.62	0.00	0.00	0.00	0.00

results (Table VI). All the solutions of alcohol produced a decrease in rate of photosynthesis, if we assume the rate of oxidation to be constant. That caused by the 1 per cent solution was so slight as to be almost within the range of possible error, but the results given are typical of those obtained in repeated experiments. No photosynthesis occurred in the 6 and 15 per cent concentrations. In these two solutions, chlorophyll diffused out of the leaves and colored the medium appreciably. Microscopic examination showed that the chloroplasts were

TABLE VI.

Photosynthesis of Elodea in Solutions of Alcohol, Ether, Chloroform, and Chloretone.

Measured in cc. of thiosulfate employed in testing the oxygen content of the solutions. Rate as compared with photosynthesis in distilled water plus $NaHCO_3$ (pH = 7.6). Immersed for two successive 1 hr. periods.

Solution.	Concentration.	Thiosulfate.	Change.	Rate of change.
	per cent	*cc.*	*cc.*	
Water.		2.25–4.28	2.03	
Alcohol.	0.05	2.25–4.20	1.95	0.96
"	0.05	2.25–4.09	0.84	0.91
Water.		2.25–4.25	2.00	
Alcohol.	1.0	2.25–3.82	1.63	0.80
"	1.0	2.25–3.70	1.45	0.73
Water.		2.25–4.25	2.00	
Alcohol.	1.5	2.25–3.66	1.41	0.69
"	1.5	2.25–3.44	1.19	0.58
Water.		2.25–4.21	1.96	
Alcohol.	3.0	2.25–3.10	0.85	0.44
"	3.0	2.25–2.25	0.00	0.00
Water.		2.25–4.25	2.00	
Alcohol.	6.15	2.25–2.25	0.00	0.00
Water.		2.25–4.25	2.00	
Ether.	1.5	2.25–3.40	1.15	0.57
"	1.5	2.25–3.20	0.95	0.42
Water.		2.25–4.24	1.99	
Ether.	3.0	2.25–2.59	0.34	0.17
"	3.0	2.25–2.25	0.00	0.00
Water.		2.25–4.37	2.12	
Chloroform.	0.05	2.25–3.35	1.10	0.52
"	0.05	2.25–3.45	1.20	0.57
Water.		2.25–4.30	2.05	
Chloretone.	0.05	2.24–4.27	2.03	1.00
"	0.05	2.24–4.30	2.06	1.00
Water.		2.25–4.33	2.18	
Chloretone.	0.1	2.24–4.32	2.08	1.00
"	0.1	2.25–4.32	2.07	1.00

considerably smaller and lighter in color. Table VII gives the sizes of the chloroplasts, computed on the basis that 1 is normal.

Ether, in all the concentrations employed, caused an increase in the rate of respiration (Table I and Fig. 5), but the 8 per cent solution produced less initial increase than any of the others.

TABLE VII.

Size of Chloroplasts.

Measured by means of camera lucida drawings after the plant had been immersed for 3 hrs. in solutions of alcohol, ether, chloroform, and chloretone.

Solution.							
Water.	Size (average control)	6.61	6.59	6.59	6.00		
Alcohol.	Concentration, per cent	**0.05**	**1.00**	**1.50**	**3.00**	**5.00**	**15.00**
	Size*	6.67	6.62	6.57	6.26	3.42	2.91
	Ratio to normal	1.00	1.00	0.99	0.79	0.52	0.44
Ether.	Concentration, per cent	**1.50**	**3.00**	**5.00**	**8.00**		
	Size*	5.02	4.50	3.71	2.32		
	Ratio to normal	0.76	0.68	0.56	0.36		
Chloroform.	Concentration, per cent	**0.05**	**0.15**	**0.30**			
	Size*	6.03	3.58	2.47			
	Ratio to normal	0.91	0.54	0.37			
Chloretone.	Concentration, per cent	**0.05**	**0.10**				
	Size*	6.61	6.58				
	Ratio to normal	1.00	0.99				

*Size indicated in units on micrometer scale.

In this anesthetic, as in alcohol, the greatest change in the pH of the medium was caused by the most concentrated solution (8 per cent, Table III and Fig. 6). In both the 5 and 8 per cent solutions chlorides diffused from the plant with such rapidity as to indicate that the cells must be injured (Table IV and Fig. 7). Microscopic examination showed that protoplasmic rotation had

stopped completely in the 5 per cent solution at the end of 20 minutes, and in the 8 per cent solution at the end of 10 minutes (Table V and Fig. 8). Both were irreversible. In the 8 per cent

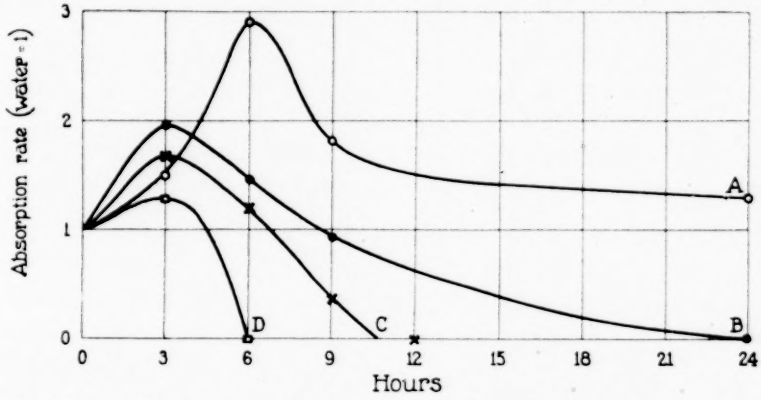

FIG. 5. Relative rate of O_2 absorption of *Elodea* in solutions of ether. Curve A, 1.5 per cent; B, 3 per cent; C, 5 per cent; D, 8 per cent.

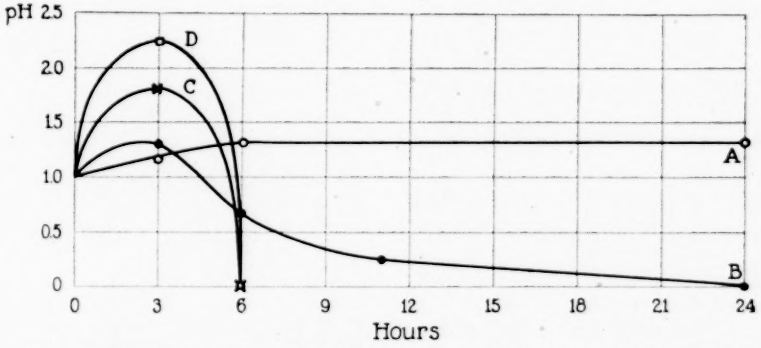

FIG. 6. Relative change of pH of solutions of ether in which *Elodea* had been kept for 3 hour periods. Curve A, 1.5 per cent; B, 3 per cent; C, 5 per cent; D, 8 per cent.

solution the cells were plasmolyzed (false plasmolysis), and after 24 hours immersion disintegration of the cell structures had set in. In the 5 per cent solution most of the cells were plasmolyzed after 2 hours. The relative size of the chloroplasts after 3

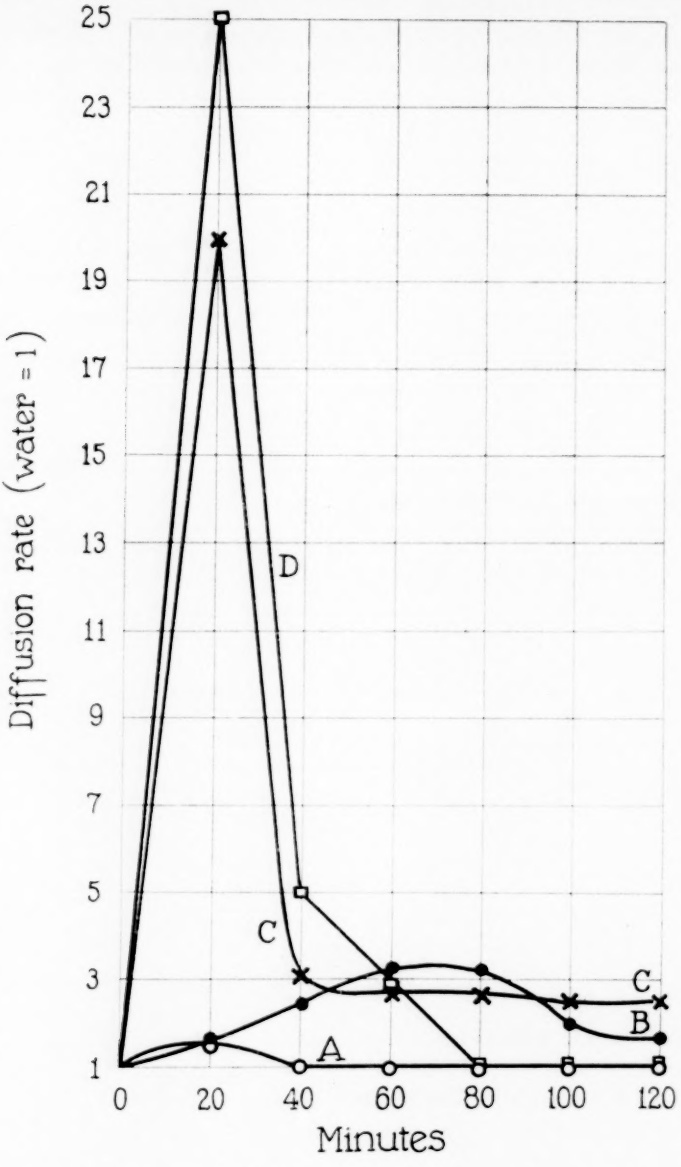

FIG. 7. Diffusion of chlorides from *Elodea* in solutions of ether during six 20 minute periods. Curve A, 1.5 per cent; B, 3 per cent; C, 5 per cent; D, 8 per cent.

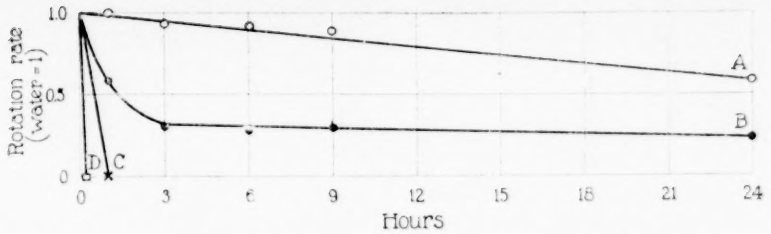

FIG. 8. Rate of protoplasmic rotation in *Elodea* in solutions of ether. Curve A, 1.5 per cent; B, 3 per cent; C, 5 per cent; D, 8 per cent.

hours in the various concentrations of ether is shown in Fig. 9, Nos. 2 to 5.

In the experiments with chloroform the differences between rates of respiration as determined by O_2 consumption and by change of pH are similar to those which occurred in alcohol. In the most concentrated solution (0.3 per cent) the greatest change in pH occurred, while O_2 consumption was less than that in the

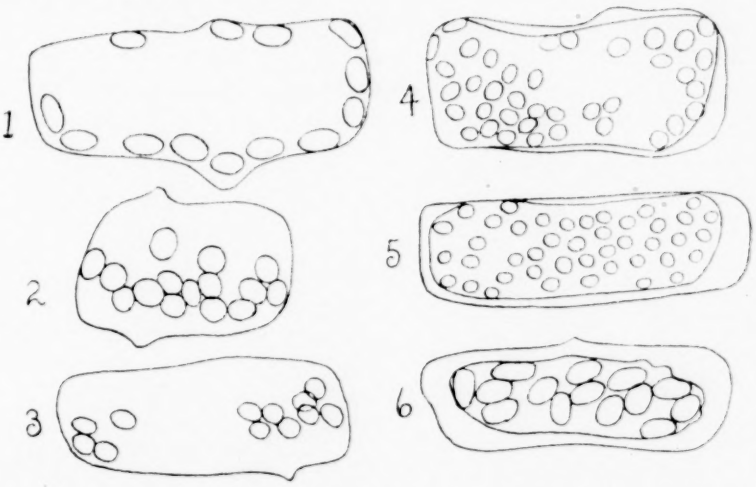

FIG. 9. Camera lucida drawing of *Elodea* cells. No. 1, normal cell; No. 2, cell exposed 2 hours to 1.5 per cent ether; No. 3, cell exposed 2 hours to 3 per cent ether; No. 4, cell exposed 2 hours to 5 per cent ether; No. 5, cell exposed 2 hours to 8 per cent ether; No. 6, cell exposed 24 hours to 0.1 per cent chloretone.

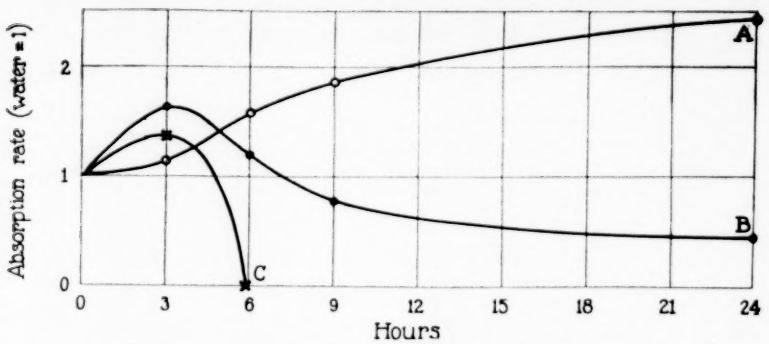

FIG. 10. Rate of O_2 absorption of *Elodea* in solutions of chloroform. Curve A, 0.05 per cent; B, 0.15 per cent; C, 0.30 per cent (Table I).

1.5 per cent solution (Tables I and III and Figs. 10 and 11). Rate of diffusion of chlorides from the cell was increased in all the solutions (Table IV and Fig. 12), but in none of them did it reach as high a figure as with alcohol or ether. In the 0.3 per cent solution exosmosis practically stopped after immersion for 40 minutes, showing that diffusion does not continue very long after the death of the cell.

Rotation stopped after immersion for 2 minutes in 0.3 per cent chloroform (Fig. 13, Curve C), but when the plant was returned

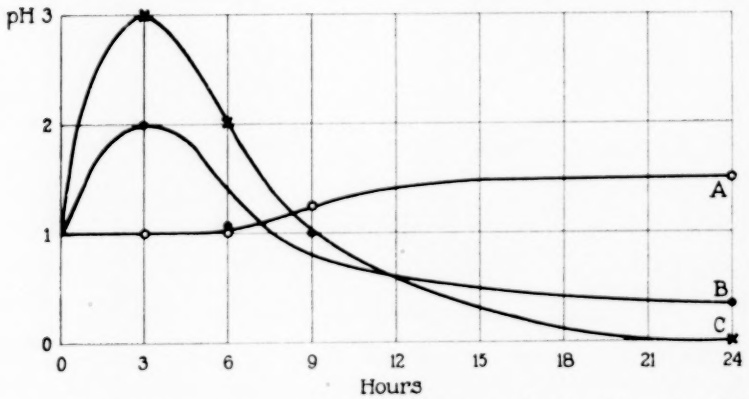

FIG. 11. Relative change of pH of solutions of chloroform in which *Elodea* had been immersed for 3 hour periods. Curve A, 0.05 per cent; B, 0.15 per cent; C, 0.30 per cent.

to water it reappeared at the end of 2 minutes. After 4 minutes in 0.3 per cent chloroform it required 45 minutes for rotation to return to normal, and after being immersed for 10 minutes the cells were irreversibly plasmolyzed and the pigment was largely

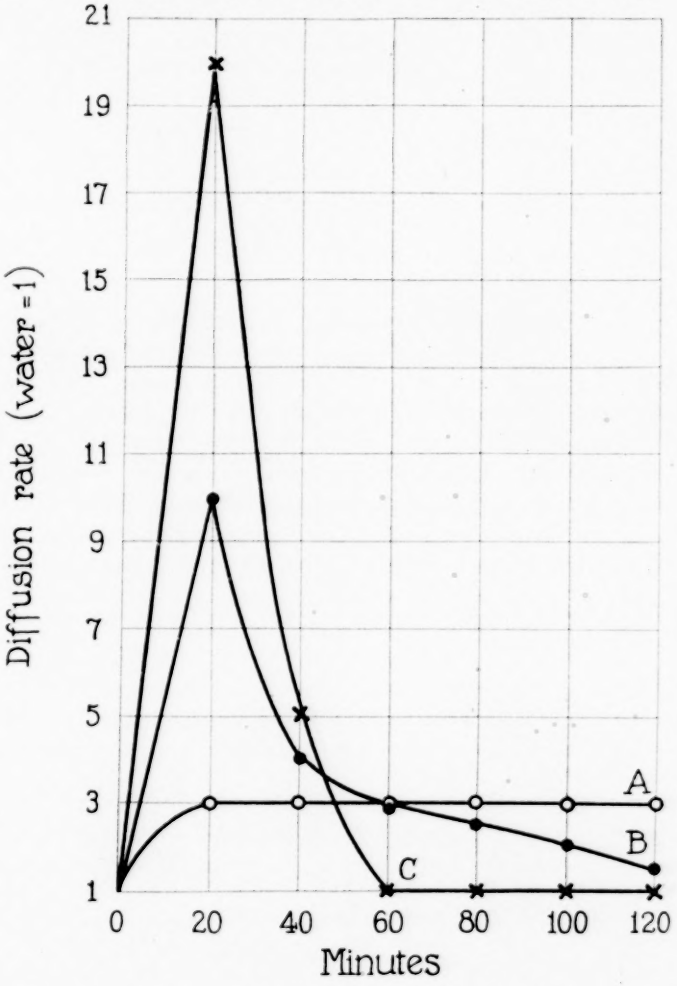

FIG. 12. Diffusion of chlorides from *Elodea* in solutions of chloroform for six 20 minute periods. Curve A, 0.05 per cent; B, 0.15 per cent; C, 0.30 per cent.

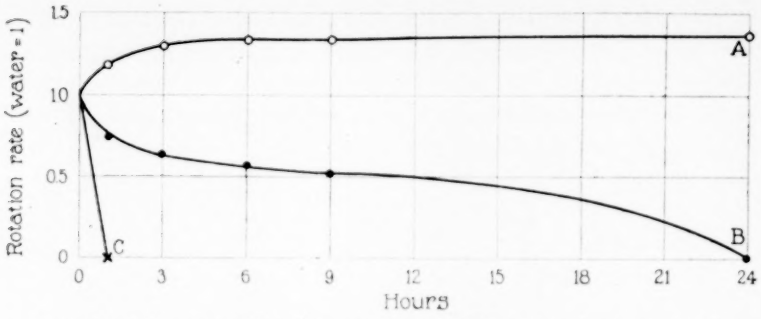

FIG. 13. Rate of protoplasmic rotation in *Elodea* in solutions of chloroform. Curve A, 0.05 per cent; B, 0.15 per cent; C, 0.30 per cent.

extracted from the chloroplasts. In both 0.15 (Curve B) and 0.3 per cent solutions of chloroform no photosynthesis occurred. In the latter, after 10 minutes immersion, photosynthesis did not reappear when the plant was returned to tap water, but in the 0.15 per cent solution photosynthesis reappeared even after 9 hours immersion.

In 0.1 per cent chloretone (Fig. 14, Curve B) respiration, measured by O_2 consumption, decreased immediately to a very low level, less than 25 per cent of the normal, but was completely reversible, resuming its original rate within 30 minutes after

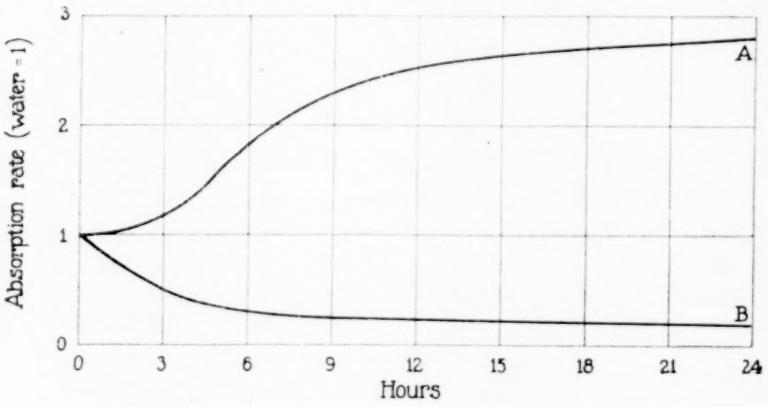

FIG. 14. Relative rate of O_2 absorption by *Elodea*. Curve A, 0.05 per cent chloretone; B, 0.1 per cent chloretone.

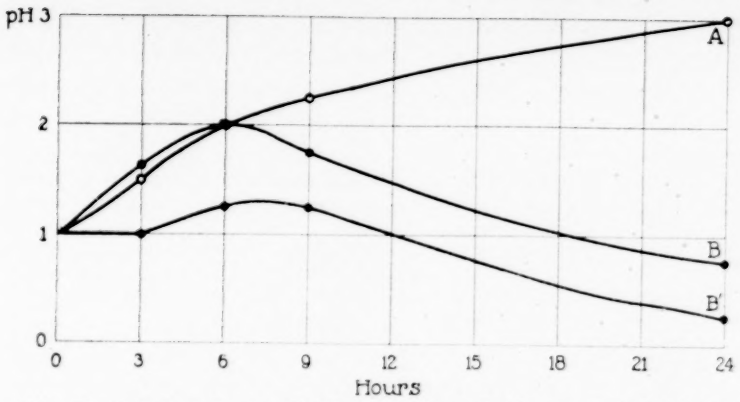

FIG. 15. Relative change of pH of solutions of chloretone during 3 hour periods. Curve A, 0.05 per cent; B, 0.1 per cent; B′ is from another experiment with plant not growing rapidly.

being returned to tap water. CO_2 production in 0.1 per cent chloretone (Fig. 15, Curve B) increased at first and then gradually fell below the normal. When tips of *Elodea* were growing less rapidly, the acceleration was much less marked, and the level reached after 24 hours considerably lower, as shown in Fig. 15, Curve B′, where it was 25 per cent of the normal. Even in this case, however, the condition was reversible. In cases of still less vigorous stalks, no acceleration took place, and these also were apparently uninjured by the anesthetic. Table VIII gives an example.

After 24 hours in 0.1 per cent chloretone the cells usually were more or less plasmolyzed (Fig. 9, No. 6). The chloroplasts, although of normal size, showed no rotation and did not change

TABLE VIII.

Time.	Reaction in water.	Reaction in 0.1 per cent chloretone solution
min.	*pH*	*pH*
0	7.80	7.65
30	7.75	7.65
60	7.70	7.60
120	7.60	7.55
180	7.50	7.45
360	7.20	7.40

position when the plant was placed in strong sunlight. Within 1 hour after returning to tap water, however, all trace of anesthesia disappeared. Fig. 16 shows exosmosis of chlorides, and Fig. 17 rate of protoplasmic rotation, under the influence of the chloretone solutions.

In Table IX are tabulated the ratios of the various cell activities. Figures for O_2 consumption, change in pH by CO_2 produc-

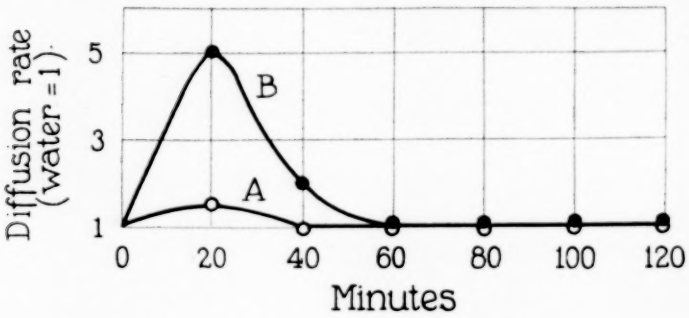

FIG. 16. Diffusion of chlorides during six 20 minute periods. Curve A, 0.05 per cent; B, 0.1 per cent chloretone.

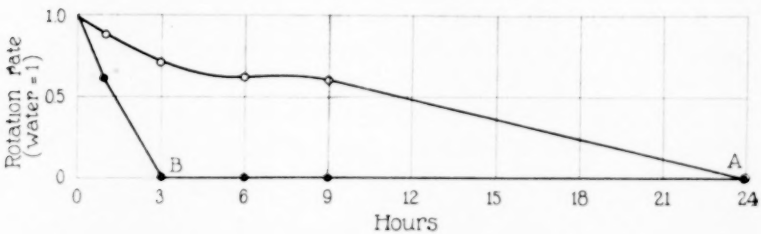

FIG. 17. Rate of protoplasmic rotation in solutions of chloretone. Curve A, 0.05 per cent; B, 0.1 per cent.

tion, protoplasmic rotation, and size of chloroplasts are taken from Tables I, III, V, and VII, respectively. Time of immersion in each case was 3 hours. Since the data in Tables IV and VI on photosynthesis and diffusion of chlorides were obtained after shorter intervals, the figures presented here have been taken from other experiments in which the period of treatment was for 3 hours.

The only solutions which did not cause increase in rate of respiration, as measured by the O_2 consumption, were 15 per cent alcohol and 0.1 per cent chloretone. In the former the plant was killed almost instantly, but in the latter the inhibition was

TABLE IX.

Ratios of the Rate of the Various Cell Processes, during 3 Hrs. Anesthesia, to the Normal Condition Taken as the Standard, and Expressed as 1.

Figures under "Photosynthesis" and "Diffusion" taken from data not presented in the tables. Other data from Tables I, III, V, and VII.

Solution.	Concentration.	O_2 consumption.	pH	Rotation.	Photosynthesis.	Size of chloroplasts.	Diffusion.	Irreversible changes.
	per cent							
Water.		1	1	1	1	1	1	
Alcohol.	0.50				0.94	1.00	1.0	
	1.00	1.12	1.00+	1.00	0.75	1.00	1.0	
	1.50	1.35	1.25	1.05	0.62	0.99	1.5	
	3.00	1.89	1.40	1.35	0.44	0.79	5.0	
	6.00	1.63	1.75	0.71	0.00	0.52	20.0	
	15.00	0.83	2.00	0.00	0.00	0.44	35.0	Plasmolysis.
Ether.	1.50	1.50	1.17	0.90	0.49	0.76	2.0	
	3.00	1.86	1.33	0.30	0.12	0.68	10.0	
	5.00	1.69	1.83	0.00	0.00	0.56	30.0	Rotation stopped.
	8.00	1.29	2.25	0.00	0.00	0.36	35.0	Plasmolysis.
Chloroform.	0.05	1.13	1.00	1.30	0.47	0.91	15.0	
	0.15	1.63	2.00	0.00	0.00	0.54	20.0	Plasmolysis.
	0.30	1.38	3.00	0.00	0.00	0.37	25.0	Photosynthesis stopped.
Chloretone.	0.05	1.17	1.50	0.71	1.00	1.00	1.5	
	0.10	0.50	1.62	0.00	1.00	0.99	7.5	

completely reversible. Hence no general rule seems to hold for all the anesthetics employed. In all cases the change of hydrogen ion concentration due to CO_2 production increased in higher concentrations of the anesthetic, and was greatest in the cells that were injured beyond recovery. In the 6 per cent alcohol,

change of pH was increased to 1.75 times the normal for the first 6 hours (Table III). These cells showed some signs of injury; when the tip of *Elodea* was not very vigorous, the leaves were killed, and even in rapidly growing tips some of the less vigorous cells were irreversibly plasmolyzed. The 5 per cent ether caused a change of pH 1.83 times that of the control by the end of the first 3 hours, and the cells were injured beyond recovery. The 0.1 per cent chloretone caused only a slightly greater CO_2 production, as measured by the change in pH, than did the 0.05 per cent solution. The cells were markedly affected by the anesthetic, many were plasmolyzed, in all of them rotation had stopped, and the chloroplasts did not change position when the plants were placed in the sunlight.

In each anesthetic, the solution of greatest concentration caused the greatest change in pH, whereas the amount of oxygen used increased through the lower concentrations of the anesthetic, reached a maximum in the solution just failing to cause permanent injury to the cell, and then decreased in those causing irreversible changes. Such an effect indicates that in respiration at least two separate processes must be involved. It is possible that the cells in strong concentrations of anesthetics more or less completely lose their power to take up oxygen (due to injury).

The 0.1 per cent solution of chloretone was the only one in which rotation was completely stopped before the cell was irreversibly injured. In all cases, however, the leaf cells seemed to react to the anesthetic much more readily than the stem cells, so that there was considerable variation as to this point even in a single leaf. In the 3 per cent ether and the 0.15 per cent chloroform, for instance, most of the movement was confined to the cells of the midrib. The leaf cells, however, recovered upon being returned to tap water.

Photosynthesis seems to be the most readily affected of any of the cell processes by alcohol, ether, and chloroform; but as these are all well known solvents of chlorophyll, such a result is probably to be expected. Decrease of size of the chloroplasts was increasingly evident in the more concentrated solutions of the three anesthetics, and it may be significant that in each case photosynthesis stopped when the chloroplasts became reduced to about one-half their normal size. In the 5 per cent

ether the cells were killed; but in the 6 per cent alcohol and the 0.05 per cent chloroform the change was reversible.

Diffusion of chlorides from the cell was enormously increased in all those cases where the plant was injured beyond recovery; *e.g.*, in 15 per cent alcohol, thirty-five times that in water; in 5 per cent ether, thirty times that in water; in 8 per cent ether, thirty-five times that in water; and in 0.3 per cent chloroform, twenty-five times that in water. Both 6 per cent alcohol and 0.15 per cent chloroform caused diffusion of chlorides twenty times that of the control. These two solutions seemed to produce about the same degree of change in all the cell processes and to leave the cell afterwards in a very similar condition. In those cases where rate of diffusion was ten times the normal or less, no permanent injury resulted.

Experiments on the Marine Jellyfish, Cassiopea.

In determining the rate of respiration, four jellyfish with manubria and nerve centers removed (15 cm. in diameter) were placed in sea water in a tightly sealed jar of 1 liter capacity and rotated in a thermostat for 1 hour in the total absence of light (to prevent photosynthesis by symbiotic plant cells). Control of temperature and O_2 content of the medium from the moment of starting the experiment was accomplished in the following manner. The thermostat was very large and was filled with fresh sea water heated quickly to the temperature of 30° by a special device (the sea being at 28–29°). The pH was rapidly determined colorimetrically and the jellyfish and jar were placed in the thermostat. The bottle containing the sample of water was sunk in the thermostat, the jellyfish were quickly placed in the jar, and the rotation was started. The water sample bottle was removed and its contents were titrated with thiosulfate, after which it was filled with pure mercury, and a two-hole rubber stopper with two tubes was inserted in place of the glass stopper. At the end of the hour the jar was opened and one of the tubes from the sample bottle inserted to the bottom. On inverting the bottle, the mercury ran out of one tube and thus sucked in water from the bottom of the jar through the other, without gaseous exchange with air. Since the jellyfish remained in the

jar during the operation, and respiration continued, extreme rapidity had to be employed in filling the bottle. The sample for pH determination was merely dipped out of the jar in a beaker, as it was found that any change that might occur through momentary contact with air was incapable of being measured under these conditions, in which the difference of CO_2 tension in the water and air was a small fraction of a millimeter of mercury.

Owing to the fact that the Tortugas Laboratory closed unusually early, the experiments were confined to the effects of ether and of CO_2. It was found that the neuromuscular system of the umbrella of *Cassiopea* was prevented from responding to electrical stimuli by the addition of 0.5 per cent or more ether to the medium. The respiratory quotient was found to be about 0.95, and, since the oxygen data are more accurate than the CO_2 determinations, only the former are given. In no case was the O_2 concentration at the end of an experiment reduced more than 50 per cent in the medium (5 cc. per liter at the beginning of experiment and more than 2.5 cc. at the end).

It had previously been shown that the rate of O_2 consumption depended on O_2 concentration,[1] and therefore the attempt was made to have the O_2 concentration the same at the beginning of all experiments. This was not always realized, however. Ether was mixed with the sea water in two 10 liter glass-stoppered bottles that were filled so completely as to exclude air bubbles. As the ether dissolved, a vacuum was produced and air bubbled out of the water to fill this space. The O_2 content of the water was reduced 25 per cent (or less), which, according to previous experiments, should decrease O_2 consumption 10 per cent (or less). Since 0.1° error in temperature makes a change in O_2 consumption of about 2.5 per cent and the error in titration may be 3 per cent, and since a small error occurs in taking the water samples, the probable error of an experiment may be nearly 10 per cent. It would be superfluous, therefore, to apply corrections of less than 10 per cent for decreased O_2 tension. In Table X the figures in bold-faced type have been raised 10 per cent to correct for 25

[1] McClendon, J. F., *J. Biol. Chem.*, 1917, xxxii, 291 (table). The statements to the contrary in summaries by the same author (McClendon, J. F., *J. Biol. Chem.*, 1920, xli, p. lxiv; *Year Book of the Carnegie Inst. Washington*, 1919, xviii, 203) are erroneous.

TABLE X.

Percentage of ether	0.5	1.0	1.0	1.0	2.0	2.0	3.0	4.0
O_2 used (control), *cc.*	2.4	2.4	1.8	2.7	2.2	2.1	1.2	2.1
O_2 " in ether, *cc.*	2.4	2.4	1.8	2.7	**2.0**	2.2	1.2	**1.3**
Ratio with control	1.0	1.0	1.0	1.0	0.9	1.05	1.0	0.6

per cent lowering in O_2 concentration; the pH was 8.2 at the beginning of each experiment.

The animal died in 4 per cent ether and hence the time of respiration was less than in the control and the experiment must be disregarded. In the remaining experiments, only one (in 2 per cent ether) shows a variation from the control of as much as 10 per cent. Since 10 per cent seems to be within the limit of error, the experiments do not show a change of O_2 consumption during anesthesia with ether.

CO_2 has been used to anesthetize marine animals and seems to be more effective than ether, as the latter stimulates certain cells as evidenced by the discharge of mucus. CO_2 was added to sea water in a Sparklet fountain and mixtures were made of 10, 20, 30, 40, and 50 per cent of this carbonated water with water taken directly from the sea. A correction should be made for lowered O_2 content of the carbonated water, as is done in Table XI under "corrected data."

TABLE XI.

pH	6.6	6.3	5.8	5.7	5.5
Total CO_2 per liter, *cc.*	68	73	80	82	85
O_2 used in carbonated water, *cc.*	2.0	1.9	1.6	0.7	0.4
O_2 " " " " (corrected data), *cc.*	2.0	1.9	1.8	1.0	0.5
O_2 used in control (pH = 8.2, CO_2 = 44), *cc.*	2.1	2.1	2.6	1.7	1.7
Ratio to control	0.95	0.90	0.69	0.59	0.29

It may be seen from Table XI that the progressive decrease in O_2 consumption with increase in CO_2 is certainly far beyond the limit of error of the method. The addition of the gas to sea water increases the concentration of CO_2 molecules, HCO_3 ions, and H ions. In the experiments recorded in Table XII, HCl was added to sea water to determine whether the H ions were entirely responsible for the decreased O_2 consumption. The O_2 content was not altered.

TABLE XII.

pH after adding HCl	6.6	5.8
O_2 used after HCl was added, *cc*	2.0	1.3
O_2 " in control (pH = 8.2), *cc*	2.1	1.9
Ratio to control	0.95	0.69

It may be seen that the difference between the action of CO_2 gas (Table XI) and HCl which liberates CO_2 from carbonates (Table XII) is *nil* or at least far within the limit of error of the experiments. The experiments recorded in Table XIII were made by adding enough HCl to neutralize exactly the alkaline reserve, after which we boiled off the CO_2 and increased the H ion concentration by addition of traces of phosphoric acid (not sufficient to be toxic).

TABLE XIII.

O_2 used at pH = 8.2 (control), *cc*	2.9
O_2 " " pH = 5.8 (CO_2-poor), *cc*	2.7
Ratio to control	0.93

It may be inferred from Table XIII that by changing the pH to 5.8 without increase of CO_2 respiration was not depressed (93 per cent of the control being within the limit of error of the method). This experiment was performed with the most extreme care since it was evident that time would not be available for a repetition of it. It indicates that H ions are not the only factor in depressing respiration, and the CO_2 molecules or ions are depressants. Perhaps the CO_2 inhibits respiration by acting as the end-product in a reversible reaction. The fact that respiration in *Cassiopea* varies directly with O_2 concentration indicates that oxidation is a simpler phenomenon in this animal than in those organisms in which CO_2 production is more or less independent of O_2 consumption.

STUDIES IN NUTRITION.

IV. THE NUTRITIVE VALUE OF PEANUT FLOUR AS A SUPPLEMENT TO WHEAT FLOUR.

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(From the Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington.)

(Received for publication, May 28, 1920.)

Valuable studies on the milling products of wheat have been made by Osborne and Mendel (1) who have shown that commercial patent wheat flour is deficient in water-soluble vitamine as well as in the quality of its protein mixture. Voegtlin, Lake, and Myers (2) have also shown that "patent" and "highly milled" wheat flours are deficient in water-soluble vitamine and recommend the use of whole wheat flours.

As will be shown later, bread made from "war flour" which contained 74 per cent of the wheat kernel furnished sufficient water-soluble vitamine for normal growth of albino rats provided the other essentials of a complete diet were supplied. However, bread made from "war flour" lacks an efficient mixture of proteins; even when supplemented with the other non-protein ingredients of a complete diet it enables albino rats to grow at only one-third to two-thirds of the normal rate. Bread made from patent flour which is more commonly used is a still poorer food.

We have found that peanut flour is an efficient and palatable supplement to wheat flour. Peanut flour is made by grinding the press-cake which is obtained as a by-product when shelled peanuts are pressed to produce peanut oil. Such a flour contains about 7 per cent of fat and 50 per cent of proteins. The protein content of the peanut flour is therefore from four to five times as high as in wheat flour. Peanut press-cake has been used as a cattle feed and to some extent as a fertilizer because its great value as a human food has not been generally understood. The value of

the proteins of the peanut have been demonstrated by chemical work (3, 4) and by nutrition experiments performed by Daniels and Loughlin (5), Osborne and Mendel,[1] and the writers.[2] Holmes (6) has also shown that the proteins, fats, and carbohydrates of peanut flour are easily digested by human subjects. Very palatable bread can be made from wheat flour to which from 15 to 25 per cent of peanut flour has been added. Such bread is rich in water-soluble vitamine and contains a protein mixture adequate for normal growth at a cost of less than one-fifth of the cost of proteins derived from animal sources.

A bread has also been prepared which contains the proteins, salts, and vitamines needed for the normal growth of albino rats. With this it has been found possible to obtain normal growth on a diet of only bread and water.

Wheat Flour Used in Experiments.—Two types of wheat flour were used to prepare the breads described herein. One was a "war flour" containing about 74 per cent of the wheat kernel and this will be designated as Flour A. This contained 0.56 per cent of ash and 2.01 per cent of nitrogen. The second type, designated as Flour B, was especially milled for another project. It was made from Kansas hard wheat and was assembled from mill stream middlings Nos. 1, 2, 3, 4, 5, and one-half of No. 6 and sizing stocks. This flour contained 0.55 per cent of ash and 2.11 per cent of nitrogen.

Bread Used in Experiments.—Breads were made from the wheat flour alone and from mixtures of the wheat flours and peanut flour. Bread made from 25 parts of peanut flour and 75 parts of wheat flour will be designated as 25 per cent peanut bread, while that made from 15 parts of peanut flour and 85 parts of wheat flour will be called 15 per cent peanut bread.

The breads[3] were made according to the following formula.

	gm.
Flour	400
Compressed yeast	10
Granulated sugar	10
Table salt	6
Water	228

[1] Private communication.

[2] Unpublished data.

[3] The bread used in this experiment was made by Miss Pearl A. Bernhardt of the Protein Investigation Laboratory, Bureau of Chemistry.

The above quantities were used for one loaf and ten such loaves were baked each time. The salt and sugar were dissolved in the water and the solution was mixed with the yeast. This suspension was then mixed thoroughly with the flour and the resulting dough was handled in the usual manner. The peanut breads were made in the same way using the proper proportions of wheat and peanut flour and mixing these carefully. After baking, the bread was sliced, dried at 60°C., and ground to a powder which was used for preparing the diets.

Calculation of the Protein Content of the Breads.—The percentage of protein in the bread was calculated by multiplying the percentage of nitrogen found by 5.7. This is the factor used for gliadin in wheat and is also nearly the factor for converting nitrogen to protein in the peanut proteins (3).

Growth Obtained on Wheat Bread.—Experiments were performed with bread made wholly from either Flour A or B as previously described. The diets were composed of 80 parts of dried bread, 16 parts of butter fat, and 4 parts of a salt mixture.[4] The rats fed on the diet containing bread from Flour A grew approximately one-third to two-thirds of the normal rate. Of six rats used four were kept on this diet for from 270 to 315 days when the experiment was discontinued. One of the other rats began to decline after growing for about 160 days. Another one died at the end of 160 days. The growth curves are shown in Charts 1 and 2.

The failure of the rats to grow normally was not due to a deficit of water-soluble vitamine since normal growth was obtained when 12 per cent of purified casein replaced an equivalent amount of bread in the diet. Since yeast contains water-soluble

[4] The composition of the salt mixture used in rations described herein is the same as that used by Osborne and Mendel in their experimental diets. See Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 374.

	gm.		*gm.*
$CaCO_3$	134.8	Citric acid + H_2O	111.1
$MgCO_3$	24.2	Fe citrate + $1\frac{1}{2}$ H_2O	6.34
Na_2CO_3	34.2	KI	0.020
K_2CO_3	141.3	$MnSO_4$	0.079
H_3PO_4	103.2	NaF	0.248
HCl	53.4	$K_2Al_2(SO_4)_4$	0.0245
H_2SO_4	9.2		

vitamine none was incorporated in the bread used for this experiment. The growth curves obtained are shown in Chart 3.

Growth Obtained on 25 Per Cent Peanut Bread.—Two different diets were prepared from this bread, one containing from 16.3 to

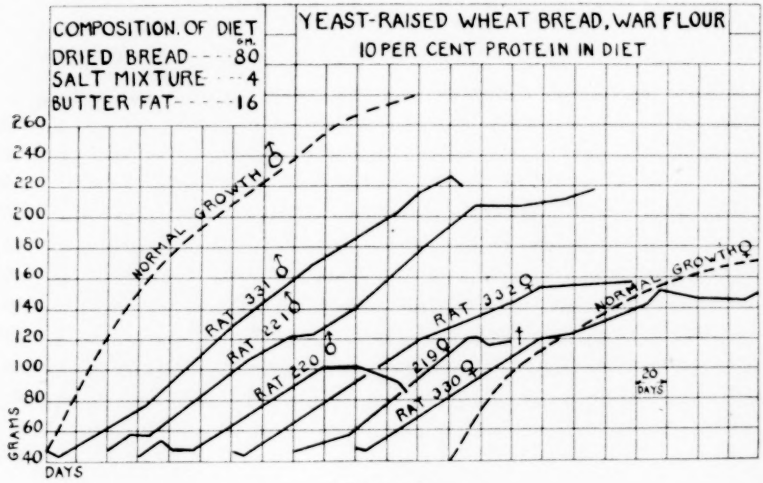

CHART 1.

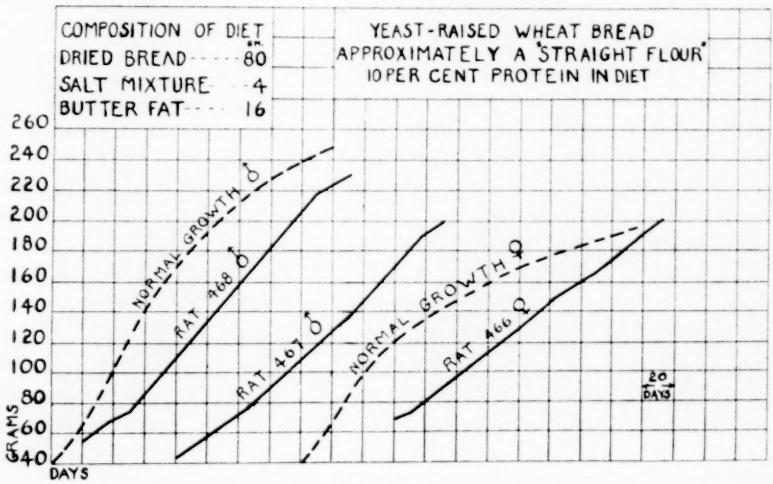

CHART 2.

16.8 per cent and the other 10 per cent of protein. The rats fed on the diets containing 16.3 or 16.8 per cent of protein grew normally. The composition of the diet and the growth curves

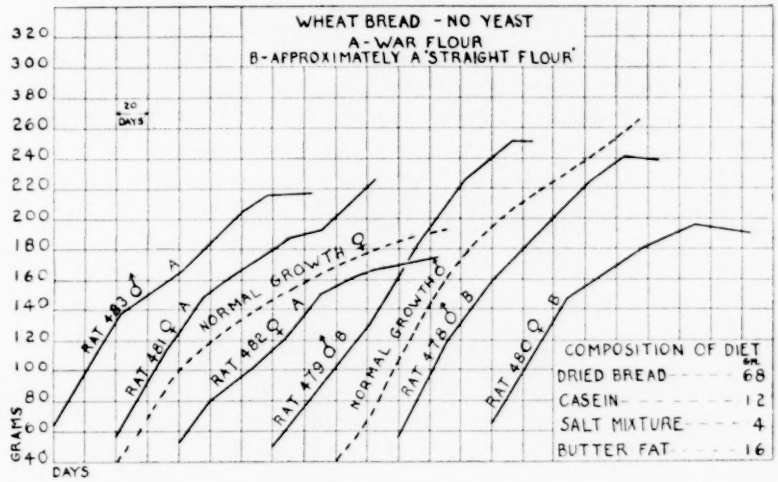

Chart 3.

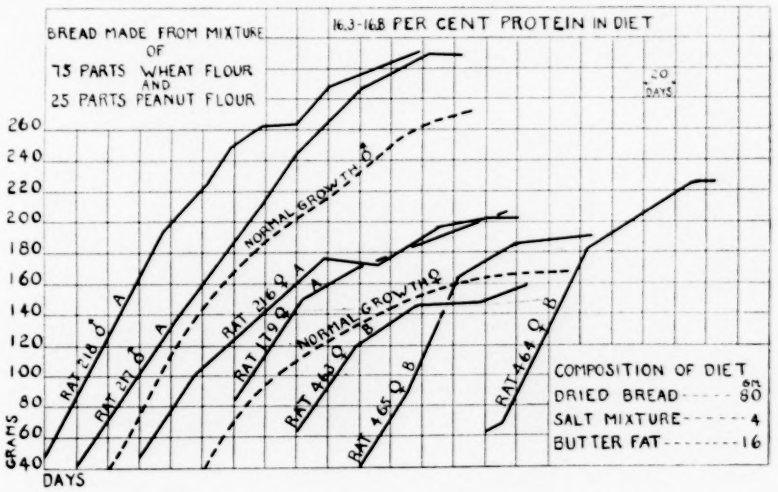

Chart 4.

are shown in Chart 4. The diet containing 10 per cent of protein was prepared by replacing a part of the bread with starch. This also produced normal growth but with a slight retardation during the first few weeks. The results are shown in Chart 5.

The excellent growth obtained on this diet containing but 10 per cent of proteins derived from bread made with a mixture of peanut flour and wheat flour in contrast with the poorer growth on a wheat bread diet which also contained 10 per cent of proteins, shows clearly the superiority of the peanut bread.

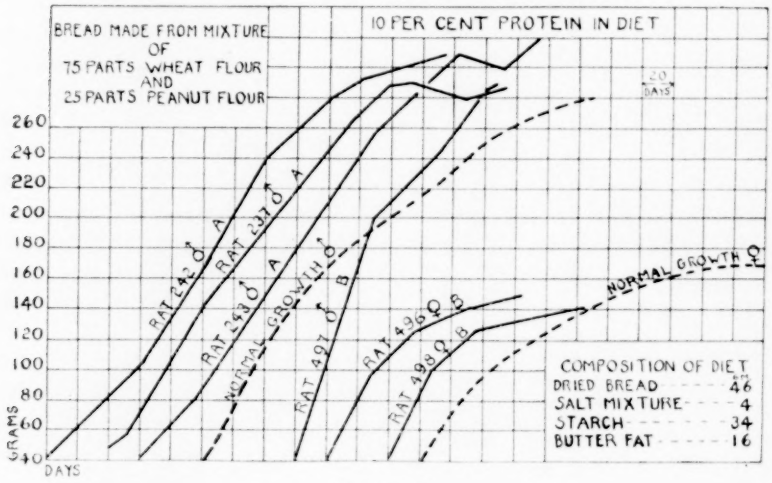

Chart 5.

Growth Obtained on 15 Per Cent Peanut Bread.—A diet was prepared from this bread and contained 14 per cent of protein. The rats fed on this diet grew at almost a normal rate as shown by the curves in Chart 6.

Efficiency of the Proteins in the Different Breads.—During the course of these experiments the food intakes were recorded in order to establish index numbers that would give the ratio of the gains in weight to the grams of protein ingested.

In doing this we have followed the procedure suggested by Osborne, Mendel, and Ferry (7). It is to be understood that the ratios obtained by us give only approximate results. The diets varied somewhat in calorific value which influenced the quantity

of food ingested. The rates at which different rats grow vary considerably and more accurate results could have been obtained by using more animals in each of the experiments. We think, however, that the comparative data given in Tables I and II show that a mixture of wheat and peanut flour furnishes more efficient proteins than those found in wheat flour alone. It is also apparent that the proteins of the peanut breads are much better utilized than those in the wheat bread.

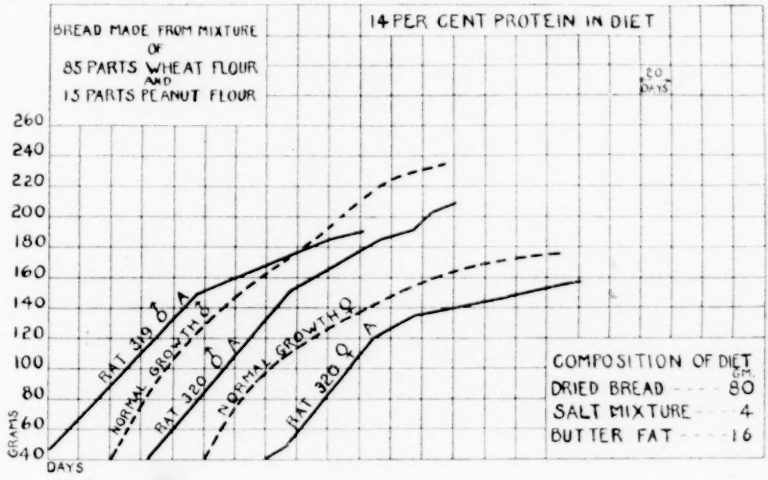

CHART 6.

SUMMARY.

1. A diet containing bread made from wheat flour (74 per cent extraction) when fed to albino rats as the only source of protein and water-soluble vitamine, together with an adequate inorganic salt mixture and butter fat, produced only about one-third to two-thirds of normal growth.

2. Bread made with a mixture of 25 parts of peanut flour and 75 parts of wheat flour furnished adequate proteins and water-soluble vitamine for normal growth. A similar bread containing 15 parts of peanut flour and 85 parts of wheat flour contained proteins and sufficient water-soluble vitamine for growth at very nearly the normal rate.

TABLE I.

Gain of Body Weight Per Gm. of Ingested Protein Furnished by 15 and 25 Per Cent Peanut Bread and Wheat Bread (War Flour).

Source of protein.	Protein in food.			Rat.	Initial body weight	Gain in 4 wks.	Gain in 10 wks.	4 wks. period.					10 wks. period.				
								Total intake.		Intake per gm. of gain.		Gain per gm. of protein.	Total intake.		Intake per gm. of gain.		Gain per gm. of protein.
	From wheat flour.	From peanut flour.	Total protein in diet.					Food.	Protein.	Food.	Protein.		Food.	Protein.	Food.	Protein.	
	per cent	*per cent*	*per cent*		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
25 per cent peanut bread.	6.8	9.5	16.3	216♀	47	48	95	205	33.4	4.3	0.70	1.44	545	88.8	5.73	0.93	1.07
				217♂	42	38	108	175	28.5	4.6	0.75	1.33	498	81.2	4.62	0.75	1.33
				218♂	48	57	138	213	34.7	3.7	0.61	1.64	614	100.1	4.45	0.73	1.38
Average										4.2	0.69	**1.47**			4.93	0.80	**1.26**
25 per cent peanut bread.	4.2	5.8	10.0	242♂	42	27	84	218	21.8	8.1	0.81	1.24	582	58.2	6.93	0.69	1.44
				243♂	43	28	99	200	20.0	7.1	0.71	1.40	597	59.7	6.03	0.60	1.66
				237♂	48	30	100	214	21.4	7.1	0.71	1.40	673	67.3	6.73	0.67	1.48
Average										7.4	0.74	**1.35**			6.56	0.65	**1.53**
15 per cent peanut bread.	8.1	5.9	14.0	318♀	42	28	82	153	21.4	5.5	0.76	1.31	473	66.2	5.77	0.81	1.24
				319♂	52	24	73	169	23.7	7.0	0.99	1.01	446	62.4	6.12	0.85	1.17
				320♂	39	26	87	150	21.0	5.8	0.81	1.24	473	66.2	5.44	0.76	1.31
Average										6.1	0.85	**1.19**			5.78	0.81	1.24

Wheat bread.	10	0	10.0	219♀	47	10	36	144	14.4	14.4	1.44	0.69	394	39.4	10.9	1.09	0.91
				220♂	45	3	24	146	14.6	48.7*	4.87*	0.21*	353	35.3	14.7	1.47	0.68
				221♂	48	10	42	159	15.9	15.9	1.59	0.63	412	41.2	9.8	0.98	1.02
				330♀	49	9	36	151	15.1	16.8	1.68	0.60	382	38.2	10.6	1.06	0.94
				331♂	47	8	46	155	15.5	19.4	1.94	0.52	376	37.6	8.2	0.82	1.22
				332♀	46	12	38	155	15.5	12.9	1.29	0.77	392	39.2	10.3	1.03	0.97
Average										15.9	1.59	**0.64**			10.8	1.08	**0.96**

* Not counted in average.

TABLE II.

Gain of Body Weight Per Gm. of Ingested Protein Furnished by 25 Per Cent Peanut Bread and Wheat Bread (Approximately a "Straight Flour").

Source of protein.	Protein in food.			Rat.	Initial body weight.	Gain in 4 wks.	Gain in 10 wks.	4 wks. period.					10 wks. period.				
								Total intake.		Intake per gm. of gain.		Gain per gm. of protein.	Total intake.		Intake per gm. of gain.		Gain per gm. of protein.
	From wheat flour.	From peanut flour.	Total protein in diet.					Food.	Protein.	Food.	Protein.		Food.	Protein.	Food.	Protein.	
	per cent	*per cent*	*per cent*		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
25 per cent peanut bread.	7.4	9.4	16.8	463 ♀	71	49	75	253	42.6	5.17	0.87	1.15	610	102.5	8.1	1.37	0.73
				464 ♀	68	72	124	267	44.9	3.71	0.62	1.60	707	118.8	5.7	0.96	1.04
				465 ♀	52	58	124	230	38.7	3.97	0.67	1.50	646	108.5	5.2	0.88	1.14
Average										4.28	0.72	**1.42**			6.3	1.07	**0.97**
25 per cent peanut bread.	4.2	5.8	10.0	496 ♀	40	56	94	189	18.9	3.38	0.34	2.96	545	54.5	5.8	0.58	1.73
				497 ♂	36	100	190	273	27.3	2.73	0.27	3.67	817	81.7	4.3	0.43	2.33
				498 ♀	42	57	86	205	20.5	3.60	0.36	2.78	518	51.8	6.0	0.60	1.66
Average										3.24	0.32	**3.14**			5.4	0.54	**1.91**
Wheat bread.	10	0	10	466 ♀	72	24	56	219	21.9	9.13	0.91	1.09	574	57.4	10.2	1.02	0.98
				467 ♂	50	18	50	175	17.5	9.72	0.97	1.03	519	51.9	10.4	1.04	0.96
				468 ♂	62	20	56	202	20.2	10.10	1.01	0.99	538	53.8	9.6	0.96	1.04
Average										9.65	0.96	**1.04**			10.1	1.01	**0.99**

3. Wheat flour (74 per cent extraction) contains sufficient water-soluble vitamine for the normal growth of albino rats.

4. The proteins in the peanut bread were utilized almost twice as well as those contained in the wheat bread.

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A METABOLIC STUDY OF AMYOTONIA CONGENITA.

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Plates 5 and 6.

(Received for publication, May 13, 1920.)

The condition of amyotonia congenita is generally defined as a disease beginning at or before birth or in early infancy, probably due to a congenital developmental defect of the lower motor neurons and of the voluntary muscles. Clinically it is characterized by weakness, hypotonia, and quantitatively diminished electrical response in the muscles, usually without disturbance of sensation or mentality. The literature is reviewed by Reuben (1) and Faber (2).

Review of the literature on the subject reveals only three references concerning the chemistry of Oppenheim's disease. Spriggs (3) published the first chemical research, making a study of the creatinine and uric acid excretion. By comparing his results obtained from an experiment with a boy suffering from Oppenheim's disease with a normal boy of the same age and practically the same weight, he showed that the creatinine excretion is diminished to less than one-half the normal figure while the uric acid remains normal. Spriggs statement concerning creatinine was confirmed in 1912 by Gittings and Pemberton (4), who also established a normal calcium retention. The case studied by Powis and Raper (5) which was associated with some nutritional disturbance and acholia was decidedly different from those cases previously mentioned. They found a low creatinine excretion associated with a high creatine excretion, a normal calcium retention, and a relatively high potassium retention.

Our case, W.H., showed the classical symptomatology described by Reuben (1) and Faber (2).

He is 3½ years old and of American nationality. He speaks somewhat slowly with a slight quaver in his voice as though there was difficulty in articulation. At 9 months he was able to sit up if helped and supported but has at no time been able to raise himself voluntarily. When sitting

up if his head falls backward he is unable to raise it again. He is inclined to be nervous, and cries easily and for long periods.

The patient appeared to be overnourished, but was flabby with a general lack of muscular tone seeming to involve all the voluntary muscles of the body. His color was good. He was bright and interested in his surroundings. A fine tremor of both hands upon the slightest exertion resulted, but there were no spasms, twitchings, choreaform movements, or convulsions. The patient was able to perform the natural movements with both arms and legs within narrow limits, but only in a weak and uncertain manner.

The hemoglobin was 94 per cent. White blood cells 10,600, red blood cells 4,800,000. Polymorphonuclear cells 41 per cent. Lymph cells 53 per cent. Large mononuclear 1. Transitional 2. Eosinophils 3. There was no response to electrical stimulus under 5 milliamperes. A small piece of muscle was obtained and sections were cut in paraffin. Only a few fibers of normal size were found in the sections. These occurred for the most part in a single group but a few were scattered throughout the section. Nearly all the fibers were greatly reduced in size, the diameter being usually about one-tenth that of the normal. Many muscle fasciculi contained only atrophic fibers. The fibers showed no abnormality except a decrease in size and an indistinctness of the fibrillæ. There was no hyaline degeneration or necrosis. The relative size and the arrangement of the atrophic fibers may be seen in the photomicrographs (Figs. 1, 2, and 3), each of which contains one or more normal fibers.

Treatment.—The patient has had cod liver oil and phosphorus over long periods previous to metabolic study, and has had a thorough test on thyroid and a mixture of thyroid and pituitary glands without any definite improvement. Under careful diet with rather large amounts of protein, with oil rubs, expert massage, and carefully executed regular exercise, and with the support of a light body brace, the patient has shown some slight improvement in muscular status. He suffered an acute attack of pneumonia in March, 1919, but made a normal recovery with no marked change in his general condition.

During the experimental period, which lasted a month, W. H. was kept on a creatinine-free diet. This period which commenced and ended with a fasting period was followed by a dose of charcoal. The method of McCrudden (6) for performing metabolism experiments was rigidly adhered to throughout by a special nurse. The food, feces, and urine were collected for 24 hour periods from 6 a.m. to 6 a.m. The urine was analyzed daily except for January 25th to 26th, 27th to 28th, and February 8th to 9th. On those days the specimens for January 25th to 26th, 27th to 28th, and February 8th to 9th were mixed and the daily average was taken. The feces, which were combined in periods of 3 and 4 days, were analyzed and the average was taken. The food analyses were also made in the same way. On the day the 24 hour period ended, the urine analyses were made, the food and feces made acid with hydrochloric acid, and these were put on the water bath to dry.

Methods of Analysis.

Acetone	Rothera's qualitative, Messinger and Hupper's quantitative.
Acidity	Folin.
Ammonia	Folin macro-aeration.
Ash	Ashing dry solids in muffle.
Chlorides	Volhard-Arnold.
Creatine-creatinine	Folin.
Fat	Soxhlet extraction.
Nitrogen total	Kjeldahl.
Phosphates	Uranium acetate.
Protein	Heller's ring.
Solids total	Drying method.
Sugar	Benedict.
Sulfates	Folin.
Urea	Plimmer and Skelton.
Uric acid	Benedict and Hitchcock.

The volume of the urine (Table I) was reduced to about one-half the normal volume for a child 2 to 3 years of age. This was due to the fact that the child was largely on a vegetable diet and perspired a great deal. To avoid any increase or variation in the nitrogenous elimination by flushing the tissues (7) the fluid intake was kept as high and as nearly constant as possible. As W. H. had no desire for food, it was very difficult to get him to eat or drink. Sometimes $1\frac{1}{2}$ hours were required to feed him a simple meal. Several weeks before this experiment was started he was fasted for 2 days, but even after that he showed no craving for food.

The urine was always acid, and contained no protein or sugar. The indican test was negative, hence the intestinal putrefaction was normal or else no tryptophane or indole was formed.

Acetone was rarely present in the urine and only in small amounts. If this condition is in any way analogous to carbohydrate starvation, an increase of acetone should be expected, at least since there is an apparent breaking down of the tissues. The absence of acetone may be due to the relatively high carbohydrate content of the diet compared with the protein and fat. On examining the urinary sediment the only thing of interest found was the calcium phosphate crystals. These were the dicalcium phosphate crystals which are common in anemia and diseases in which articulations are affected (8). The feces, which varied

TABLE I.

Urine Analyses.

Date.	Volume.	Specific gravity.	Reaction.	Protein.	Sugar.	Indican.	Acetone.	Acidity, expressed in cc. 0.1 N NaOH.	Total N.	Urea N.	NH_3 N.	Uric acid.	Creatine.	Creatinine.	Total creatine and creatinine.	Total solids.	Ash.	NaCl	P_2O_5
1919	*cc.*						*gm.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Jan. 15	255	1.030	Acid.	—	—	—	0.0859	147.68	3.988	2.667	0.227	0.168	0.051	0.263	0.307	17.76	4.812	2.520	0.736
" 16	320	1.020	"	—	—	—	—	108.80	3.506	2.879	0.164	0.168	0.068	0.124	0.183	14.72	5.250	3.490	0.605
" 17	220	1.020	"	—	—	—	—	35.00	2.700	2.128	0.161	0.145	0.046	0.084	0.125	9.98	4.208	2.829	0.373
" 20	290	1.023	"	—	—	—	—	88.64	2.872	2.082	0.159	0.186	0.110	0.084	0.180	13.99	3.856	1.344	0.608
" 21	315	1.020	"	—	—	—	—	61.40	2.822	2.226	0.149	0.159	0.111	0.082	0.178	12.47	5.120	2.479	0.507
" 22	280	1.020	"	—	—	—	—	45.12	2.464	1.854	0.137	0.099	0.074	0.074	0.138	12.45	6.064	3.497	0.483
" 23	290	1.023	"	—	—	—	—	79.20	3.028	2.365	0.136	0.178	0.136	0.091	0.219	14.03	6.550	3.372	0.588
" 24	250	1.023	"	—	—	—	—	108.96	1.803	1.352	0.102	0.145	0.065	0.112	0.168	11.58	5.024	2.644	0.564
" 25	308	1.024	"	—	—	—	—	143.28	4.122	3.368	0.102	0.210	0.143	0.103	0.227	16.20	4.734	2.165	0.843
" 26	280	1.024	"	—	—	—	—	143.28	4.122	3.368	0.102	0.210	0.143	0.103	0.227	16.20	4.734	2.165	0.843
" 27	220	1.025	"	—	—	—	—	68.54	2.028	1.258	0.182	0.122	0.095	0.085	0.167	10.99	4.291	2.074	0.390
" 28	210	1.025	"	—	—	—	—	68.54	2.028	1.258	0.182	0.122	0.095	0.085	0.167	10.99	4.291	2.074	0.390
" 29	220	1.025	"	—	—	—	—	107.52	2.791	2.198	0.202	0.202	0.115	0.088	0.187	12.20	4.048	2.376	0.568
" 31	260	1.025	"	—	—	—	0.0184	100.64	4.250	3.202	0.147	0.206	0.132	0.101	0.215	14.64	6.611	2.928	0.718
Feb. 1	260	1.030	"	—	—	—	—	111.20	3.571	2.958	0.153	0.212	0.102	0.086	0.175	15.97	5.928	3.120	0.737
" 3	240	1.030	"	—	—	—	—	116.00	2.915	2.434	0.229	0.196	0.046	0.080	0.120	14.01	4.780	2.672	0.612
" 4	220	1.020	"	—	—	—	—	62.88	2.502	1.994	0.180	0.152	0.077	0.072	0.138	9.98	4.056	2.424	0.464
" 5	270	1.023	"	—	—	—	—	74.56	2.582	2.159	0.151	0.194	0.090	0.080	0.158	11.95	5.104	2.768	0.551
" 6	140	1.035	"	—	—	—	—	60.48	2.130	1.609	0.115	0.142	0.091	0.085	0.163	8.86	3.204	1.656	0.420
" 7	160	1.035	"	—	—	—	—	79.65	2.412	1.871	0.142	0.144	0.128	0.089	0.200	12.14	4.646	1.764	0.522
" 8	325	1.019	"	—	—	—	—	65.82	2.639	2.208	0.146	0.140	0.104	0.087	0.177	15.27	5.749	2.369	0.572
" 9	230	1.029	"	—	—	—	—	65.82	2.639	2.208	0.146	0.140	0.104	0.087	0.177	15.27	5.749	2.369	0.572
" 10	425	1.016	"	—	—	—	—	84.80	3.341	2.803	0.182	0.162	0.124	0.088	0.196	13.40	6.464	3.740	0.613
" 11	190	1.035	"	—	—	—	+	128.04	3.720	3.032	0.124	0.074	0.130	0.087	0.200	13.68	4.584	1.836	0.714
" 12	210	1.033	"	—	—	—	+	137.20	3.732	3.252	0.217	0.126	0.090	0.091	0.169	15.07	5.068	2.716	0.854
" 13	135	1.033	"	—	—	—	—	59.25	2.119	1.669	0.140	0.151	0.059	0.061	0.113	9.95	3.798	2.142	0.392
" 14	280	1.025	"	—	—	—	—	60.48	3.520	2.302	0.161	0.161	0.087	0.083	0.158	14.64	7.368	3.256	0.680

TABLE II.

Nitrogen, Fat, and Ash Balances.

Date.	Volume.	Feces, dry weight.	Food, dry weight.	Total N intake.	Total N output.	Nitrogen balance.	Total fat intake.	Total fat output.	Fat balance.	Ash intake.	Ash output.	Ash balance.
1919	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Jan. 15	255	24.23	616.50	10.09	11.31	−1.22	71.86	1.79	+70.10	24.05	18.80	+5.25
" 16	320											
" 17	220											
" 20	290	21.55	651.00	10.95	9.21	+0.74	90.71	1.27	+89.44	21.19	17.85	+3.34
" 21	315											
" 22	280											
" 23	290	42.96	851.50	9.79	14.11	−4.32	138.19	4.12	+134.07	23.77	28.67	−4.90
" 24	250											
" 25	308											
" 26	280											
" 27	220	33.87	562.33	9.75	8.59	+1.16	83.70	3.42	+80.28	17.79	18.35	−0.56
" 28	210											
" 29	220											
" 31	260	21.01	355.90	8.17	8.96	−0.80	60.69	1.74	+58.95	15.12	15.32	−0.20
Feb. 1	260											
" 3	240	26.72	545.79	10.96	9.20	+1.76	82.81	2.24	+80.57	16.85	17.55	−0.70
" 4	220											
" 5	270											
" 6	140	33.88	837.90	10.40	11.63	−1.23	105.00	2.35	+102.65	21.63	24.52	−2.89
" 7	160											
" 8	325											
" 9	230											
" 10	425	32.61	426.24	9.70	12.48	−2.78	69.42	2.26	+67.16	18.59	22.26	−3.67
" 11	190											
" 12	210											
" 13	135	70.59	419.30	7.55	10.57	−3.02	48.22	6.63	+41.59	18.44	23.19	−4.74
" 14	280											

in color from light to dark brown, were neutral or slightly acid in reaction and contained hydrobilirubin.

The child weighed 13.49 kilos at the beginning of the experiment and at the end 13.21 kilos. The approximate caloric intake varied from 700 to 1,400 calories per day; the average for the period being 900 or about 53.5 calories per kilo of body weight. According to Sommerfeld (9), this is about 25 calories less per kilo than for a normal child of this age.

Although the nitrogen balance (Table II) for the entire period was negative, there were several periods during the month in which nitrogen equilibrium was maintained. For the month the loss of nitrogen was 9.7 gm.

Camerer (10) has established 0.695 gm. of P_2O_5 as normal urinary elimination for boys of 3 years of age. According to this the average daily phosphorus metabolism is normal.

The ash balance (Table II) for the period is negative.

Since protein is the source of the urinary sulfur as well as nitrogen (Tables III and IV), a parallelism, although not exact, can be expected in the daily variations of nitrogen and sulfur. The figures for neutral sulfur and undetermined nitrogen fulfill this expectation. The inorganic sulfates are slightly subnormal, while the organic and especially the neutral sulfur percentages are high, as compared with Schwarz's figures (11) for a normal 5 year boy (86 to 87 per cent inorganic sulfur, 4.0 per cent organic sulfur, and 7.8 per cent neutral sulfur). Weiss (12) thinks that most of the neutral sulfur is endogenous, also that any condition which causes a rise in the neutral sulfur can be attributed to destruction of body protein.

In the blood analysis Lyman's method (13) was used for the determination of calcium; the other methods are those described by Gradwohl (14), except that a Dubosecq colorimeter replaced the Hellige. The low urea figure which occurs in a fasting condition is the most significant (Table V). After 17 hours fast in adults Schwartz and McGill (15) found 5 to 7.1 mg. of urea N per 100 cc. of blood. Fasting also increases the blood sugar. No figures seem to be available for children of this age. Since the blood analysis applies to only one specimen of blood (sugar excepted) we do not lay stress on an interpretation of the results.

TABLE III.

Urinary Nitrogen Partition.

Date	Total N.	Urea N.	NH_3 N.	Creatine N.	Creatinine N.	Uric acid N.	Rest N.	Per cent of total nitrogen.					
								Urea N.	NH_3 N.	Creatine N.	Creatinine N.	Uric acid N.	Rest N.
1919	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Jan. 15	3.988	2.667	0.227	0.014	0.097	0.056	0.925	66.88	5.70	0.36	2.44	1.40	23.19
" 16	3.506	2.879	0.164	0.019	0.046	0.056	0.341	82.11	4.69	0.55	1.31	1.60	9.73
" 17	2.700	2.128	0.161	0.013	0.031	0.048	0.317	78.82	5.97	0.39	1.17	1.80	11.77
" 20	2.872	2.082	0.159	0.031	0.031	0.062	0.504	72.51	5.55	1.08	1.09	2.16	17.58
" 21	2.822	2.226	0.149	0.031	0.030	0.053	0.331	78.87	5.31	1.11	1.08	1.87	11.75
" 22	2.464	1.854	0.137	0.020	0.027	0.032	0.390	75.29	5.59	0.84	1.12	1.33	15.82
" 23	3.028	2.365	0.136	0.038	0.033	0.059	0.394	78.11	4.50	1.27	1.11	1.96	13.03
" 24	1.803	1.352	0.102	0.018	0.041	0.048	0.239	74.97	5.68	1.02	2.31	2.68	13.30
" 25	4.122	3.368	0.102	0.040	0.058	0.070	0.502	81.71	2.50	0.98	0.93	1.69	12.19
" 26	4.122	3.368	0.102	0.040	0.038	0.070	0.502	81.71	2.50	0.98	0.93	1.69	12.19
" 27	2.028	1.258	0.182	0.026	0.031	0.040	0.488	62.20	9.00	1.33	1.55	2.00	24.08
" 28	2.028	1.258	0.182	0.026	0.031	0.040	0.488	62.20	9.00	1.33	1.55	2.00	24.08
" 29	2.791	2.198	0.202	0.032	0.032	0.067	0.257	78.76	7.26	1.16	1.17	2.41	9.23
" 31	4.250	3.202	0.147	0.037	0.037	0.068	0.757	75.34	3.47	0.87	0.88	1.61	17.81
Feb. 1	3.571	2.958	0.153	0.028	0.032	0.070	0.327	82.84	4.29	0.81	0.90	1.98	9.17
" 3	2.915	2.434	0.229	0.013	0.029	0.065	0.143	83.51	7.87	0.45	1.02	2.24	4.91
" 4	2.502	1.994	0.180	0.021	0.026	0.050	0.227	79.70	7.23	0.86	1.08	2.03	9.10
" 5	2.582	2.159	0.151	0.025	0.029	0.064	0.151	83.62	5.87	0.99	1.16	2.50	5.85
" 6	2.150	1.609	0.115	0.025	0.031	0.047	0.300	75.55	5.44	1.21	1.48	2.23	14.08
" 7	2.412	1.871	0.142	0.036	0.033	0.048	0.281	77.58	5.91	1.49	1.37	1.98	11.64
" 8	2.639	2.208	0.146	0.029	0.032	0.046	0.176	83.67	5.56	1.11	1.23	1.77	6.67
" 9	2.639	2.208	0.146	0.029	0.032	0.046	0.176	83.67	5.56	1.11	1.23	1.77	6.67
" 10	3.344	2.803	0.182	0.035	0.032	0.054	0.236	83.82	5.45	1.04	0.98	1.61	7.07
" 11	3.720	3.032	0.124	0.036	0.032	0.024	0.468	81.52	3.35	0.99	0.87	0.67	12.60
" 12	3.732	3.252	0.217	0.025	0.033	0.042	0.161	87.12	5.83	0.68	0.91	1.13	4.33
" 13	2.119	1.669	0.140	0.016	0.022	0.050	0.220	78.75	6.60	0.79	1.08	2.38	10.39
" 14	3.520	2.302	0.161	0.024	0.030	0.053	0.947	65.40	4.59	0.70	0.88	1.51	26.92
Daily average.....	2.976	2.323	0.157	0.027	0.035	0.053	0.379	77.64	5.57	0.94	1.21	1.85	12.78

TABLE IV.

Urinary Sulfur Partition.

Date.	Total SO_3.	Inorganic SO_3.	Organic SO_3.	Neutral SO_3.	Per cent of total SO_3.		
					Inorganic SO_3.	Organic SO_3.	Neutral SO_3.
1919	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*
Jan. 15	1.385	0.769	0.149	0.467	55.52	10.75	33.72
" 16	0.702	0.602	0.018	0.081	85.75	2.56	11.54
" 17	0.495	0.427	0.002	0.066	86.26	0.41	13.22
" 20	0.589	0.435	0.035	0.118	73.85	5.94	20.03
" 22	0.521	0.394	0.020	0.106	75.62	3.84	20.34
" 23	0.687	0.565	0.034	0.087	82.24	4.94	12.66
" 24	0.695	0.597	0.029	0.068	85.90	4.17	9.78
" 25	0.846	0.738	0.037	0.070	87.23	4.37	8.27
" 26	0.846	0.738	0.037	0.070	87.23	4.37	8.27
" 27	0.585	0.506	0.033	0.044	86.49	5.64	7.52
" 28	0.585	0.506	0.033	0.044	86.49	5.64	7.52
" 31	0.781	0.652	0.041	0.087	83.49	5.25	11.14
Feb. 1	0.712	0.575	0.079	0.057	80.76	11.09	8.00
" 3	0.615	0.486	0.037	0.092	79.02	6.06	14.96
" 4	0.509	0.431	0.020	0.058	84.67	3.92	11.39
" 5	0.548	0.432	0.018	0.097	78.83	3.28	17.70
" 6	0.355	0.290	0.031	0.032	81.69	8.73	9.01
" 8	0.582	0.502	0.047	0.032	86.25	8.07	5.49
" 9	0.582	0.502	0.047	0.032	86.25	8.07	5.49
" 11	0.660	0.575	0.041	0.043	87.12	6.21	6.51
" 12	0.635	0.544	0.043	0.047	85.67	6.77	7.40
" 13	0.343	0.278	0.030	0.034	81.05	8.75	9.91
" 14	0.639	0.553	0.038	0.048	86.54	5.96	7.51
Daily average					82.34	5.86	11.63

TABLE V.

Blood Analysis.

	per cent
6–21 Sugar	0.13
7–1 "	0.14
11–27 "	0.15

	mg. per 100 cc. of blood
Creatinine	1.50
Creatine	5.45
Calcium	7.10
Urea N	3.50

SUMMARY.

The significant facts observed in the metabolism of a case of amyotonia congenita were:

1. A lowered creatinine excretion, in addition to the excretion of creatine on a low protein diet.

2. A normal uric acid excretion; therefore no nucleoprotein broken down.

3. An increased rest nitrogen, accompanied by an increased neutral sulfur.

4. A normal phosphorus excretion; therefore no bone disintegration.

5. A lowered chloride excretion.

We wish to thank Dr. J. P. Sedgwick, who suggested the problem, for his kindly interest in the research.

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EXPLANATION OF PLATES.

PLATE 5.

FIG. 1. Group of normal fibers with a number of atrophic fibers in the same fasciculus. Fasciculus of atrophic fibers on right.

PLATE 6.

FIG. 2. High power photomicrograph of Fig. 1, showing atrophic and normal fibers in the same fasciculus.

FIG. 3. Fasciculus of atrophic fibers under moderately high magnification. One normal fiber.

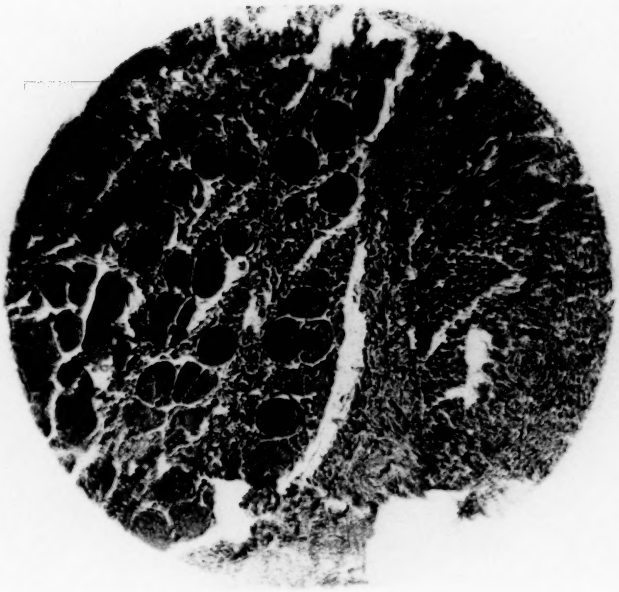

FIG. 1.

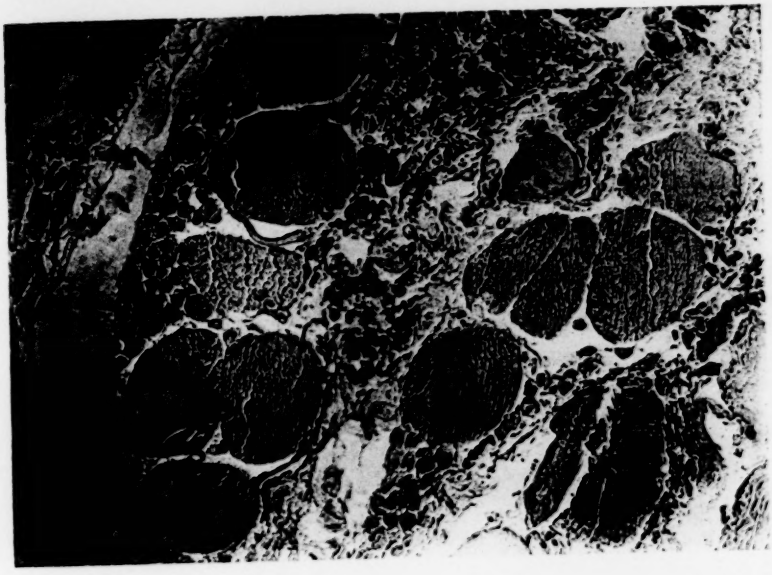

FIG. 2.

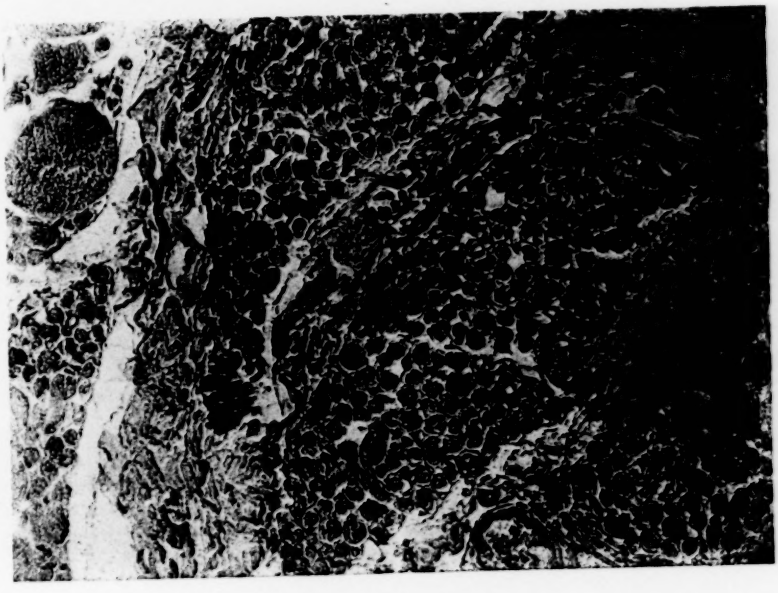

FIG. 3.

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